

**ASSESSING WATER USE EFFICIENCY AND CARBON SEQUESTRATION  
POTENTIAL OF DIFFERENT WHEAT (*Triticum aestivum*) GENOTYPES**

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## DECLARATION

I, **Nozibusiso Odette Mbava**, declare that;

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## ABSTRACT

Poor soil fertility status and limited water availability have been identified as two of the major constraints to crop production in South Africa. Under these conditions, growing crop genotypes that will sequester more carbon into the soil and be more water use efficient is crucial to improve crop production thus alleviate food insecurity. The aim of the study was to assess water use efficiency and carbon sequestration potential of different wheat genotypes. The experiment was set up under field and greenhouse conditions using 100 wheat genotypes from CIMMYT. These were grown at 25% (water-stressed) and 75% (non-stressed) field capacity (FC) using an alpha lattice with 10 blocks and 10 genotypes per block. Treatments were replicated twice in the field and three times in the glasshouse. After harvest the 10 best wheat genotypes were separated into roots and shoots, their chemical composition was analysed prior to the incubation experiment. About 0.25 g each of wheat root (RT) or shoot (ST) of the selected wheat genotypes were thoroughly mixed with 100 g of soil then transferred into an air tight PVC pot. NaOH solution was also placed inside the incubation pot to trap CO<sub>2</sub> released during decomposition, and this was measured on day 0, 7, 15, 23, 31, 39, 47, 55, 63, 77, 91, 105, and 120 of incubation. The results from the field and glasshouse experiments showed that average wheat grain yield (GY) varied from 326 g m<sup>-2</sup> to 2062 g m<sup>-2</sup>, shoot biomass (SB) ranged from 1873 g m<sup>-2</sup> to 3726 g m<sup>-2</sup> while total plant biomass (PB) ranged from 2992 g m<sup>-2</sup> to 6289 g m<sup>-2</sup>. Grain carbon stocks (GC<sub>s</sub>) averaged 132 g C m<sup>-2</sup> and 167 g C m<sup>-2</sup> in the glasshouse under stressed and non-stressed conditions, respectively. The total plant carbon stocks (PC<sub>s</sub>) ranged from 691 g m<sup>-2</sup> to 3093 g m<sup>-2</sup> (i.e. 348% difference) in the glasshouse, while they ranged from 835 g m<sup>-2</sup> to 4016 g m<sup>-2</sup> (i.e. 381% difference) in the field. Water use efficiency for grain yield production (WUE-GY) ranged from 0.12 g m<sup>-2</sup> mm<sup>-1</sup> to 2.10 g m<sup>-2</sup> mm<sup>-1</sup> (i.e. 18 fold increase) in the glasshouse under stressed conditions while it was 0.57 g m<sup>-2</sup> mm<sup>-1</sup> to 4.01 g m<sup>-2</sup> mm<sup>-1</sup> in

the field under stressed conditions. WUE components varied amongst wheat genotypes. LM75 exhibited higher WUE-GY under stressed conditions while genotypes LM48 and LM47 exhibited lower WUE-GY under non-stressed conditions. LM75 was also ranked the best genotype for WUE-PC<sub>s</sub> while BW162 was ranked the best genotype for WUE-RC<sub>s</sub>. In the incubation experiment the shoot treatments evolved higher net CO<sub>2</sub>-C compared to root treatments. Net CO<sub>2</sub>-C was highest within the first two weeks and declined with time. Amongst the root treatments, BW140 RT evolved the highest net CO<sub>2</sub>-C (86.6 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil), while LM70 RT evolved the lowest (48.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil). In shoot treatments BW162 ST and BW140 ST evolved the highest net CO<sub>2</sub>-C with average values of 218.7 and 223.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil respectively. Comparing all the 10 treatments LM70 RT evolved the lowest while BW140 ST and BW162 ST had the highest net CO<sub>2</sub>-C. The findings revealed that variability in storing C under different scenarios of water availability exists among the wheat genotypes studied. Also, the residues of different wheat residues exhibit potential of sequestering more C into the soil thus improve soil fertility.

**Keywords:** wheat, agronomic traits, grain yield, carbon stocks, carbon sequestration

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## **THESIS OUTLINE**

This thesis consists of six chapters. Chapter one is the general introduction which introduces the reader to the background of the study. Chapter two provides a quantitative review elucidating the environmental factors controlling water use efficiency at a global scale. This was done based on a comprehensive meta-analysis conducted using data from 546 water use efficiency experiments around the world published in ISI journals. This is followed by chapter three, which is the literature review based on the effect of crop residue quality on its decomposition, mineralization and C sequestration potential. Chapter four focuses on the selection of wheat genotypes for improved water use efficiency for grain yield, biomass and atmospheric carbon sequestration. This is then followed by chapter five which assess the mineralization patterns and soil carbon sequestration potential of wheat residues from different genotypes. Lastly is chapter six which is the general discussion, conclusion and recommendations of the study.

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# CHAPTER ONE

## GENERAL INTRODUCTION

### 1.1 Background and justification

Soil fertility depletion is one of the major constraints to crop production in Sub-Saharan Africa (SSA) (Sanchez et al., 1997). It is a result of soil organic matter (SOM) degradation through continuous cultivation (Van Antwerpen and Meyer, 1996), as well as depletion of soil essential nutrients (N, P, K) through processes such as leaching, erosion and increased soil acidification resulting from the application of chemical fertilizers (Aihou et al., 1998). Soil organic matter (SOM) is a rich source of mineral P, S and N in soil (Kumwenda et al., 1996), therefore its depletion will reduce the availability of these nutrients, thus reducing crop production. South Africa is characterized by top soils with very low organic matter levels, since only 4% of the top soils contain more than 2% organic carbon, 58% contain less than 0.5%, whilst 38% have between 0.5% and 2% organic carbon (Du Preeze et al., 2011).

Roberts et al. (2003) analysed ten thousand and thirty-eight (1038) soil samples from small-scale farmers in areas around Kwa-Zulu Natal (KZN). More than half of these samples were deficient in phosphorus (less than 12 mg L<sup>-1</sup>), while the remainder were severely deficient in P (less than 5 mg L<sup>-1</sup>), and 10% of the samples were severely deficient in K (less than 50 mg L<sup>-1</sup>). Smaling (1993) estimated that annual net nutrient depletion rates per hectare exceeded 30 kg N and 20 kg K in arable soils of several countries in Sub-Saharan Africa (SSA). Therefore, there is a need to improve the nutrient status of soil to enhance crop production in this region. Inorganic fertilizers have been used since the 1940's, with their use achieving considerable success over the years by increasing crop production. However, excessive use of fertilizers has been found ineffective for sustaining soil fertility, since their application may result in SOM

deterioration through soil acidification and death of beneficial microorganisms in the soil (Gruhn et al., 2000, Setboonsarng, 2006).

Organic resources have been identified as reliable alternatives to reduce continued use of inorganic fertilizers, thus improving soil fertility status of South African soils. Common organic sources in SSA include crop residues, leguminous cover crops, green manures, animal manure, mulches and household wastes (Hossner and Juo, 1999). Crop residues are amongst the common organic resources that are readily available to most farmers (Hossner and Juo, 1999). Globally, crop residue production is estimated at 3.8 billion tons per year of which 74% are from cereals, 8% from legumes, 3% from oil crops, 10% from sugar and 5% from tuber crops (Lal 2005). Incorporation of crop residues into the soil directly contributes to the build-up of soil organic matter (SOM) (Boehm and Anderson, 1997), which is crucial for improving crop yields as well as for cycling of nutrients into the soil system (Soon and Arshad, 2002) and to improve soil physical, chemical and biological properties (Kumar and Goh, 2000). As these residues decompose in the soil, they release mineral nutrients such as carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) among others (Ayuke et al., 2004, Nziguheba et al., 1998; Oguike et al., 2006).

Ibrahim et al. (2015) reported an increase of labile organic carbon fractions which were dissolved organic carbon, microbial biomass carbon, light fraction organic carbon, particulate organic carbon and permanganate oxidizable carbon after incorporating rice straw into the soil. In a study conducted by Abro et al. (2011), the addition of maize straw into the soil significantly increased soil organic carbon and microbial biomass nitrogen contents of the soil. Also, in a study conducted by Murungu et al. (2010), incorporation of grazing vetch and forage pea residues resulted in increased soil mineral N and extractable P, while incorporation of oat

residues improved soil organic matter. Therefore, the application of crop residues into the soil is beneficial as they improve soil fertility and could therefore improve crop production.

In addition to poor soil fertility, limited water availability and increased temperatures are other problems constraining crop production in Sub-Saharan Africa. Blignout et al. (2009) also indicated that climate change has led to an increase in water use in South Africa, mainly due to the increased hot and dry conditions experienced over the last decades. High temperatures have been observed to exacerbate yield reduction in some SSA crops such as maize, wheat and sorghum (Luo 2011). Wheat (*Triticum aestivum*) is one of the most important cereal crops in the world (Tunio, 2006). In South Africa, it is the second most important grain crop after maize (Gbetibouo and Hassan, 2005). Wheat is mainly used for human consumption (making of bread, biscuits, breakfast cereals etc.) as well as for seed and animal feed (DAFF, 2010). It is produced throughout South Africa, with the Western Cape and Free State provinces being the largest producers. However smaller quantities of wheat are also produced in Kwa-Zulu Natal, Eastern Cape, Gauteng and Mpumalanga provinces (DAFF, 2012).

However, wheat production in South Africa has declined steadily from 2.5 million tonnes produced on 974 000 ha in 2002, to approximately 1.7 million tonnes, produced on 500 000 ha in 2013 (Sosibo et al., 2017). This decline is a result of poor soil fertility status of South African soils. Wheat production is also hampered by low and unreliable rainfall (Gbetibouo and Hassan, 2005), as the total annual rainfall in South Africa averages about 495 mm, which is far below the world's average of 860 mm per year (FAO 2005). As a result, approximately 20% of the total area planted to wheat is under irrigation, while 80% is under rain-fed conditions (DAFF, 2012). Since predictions indicate increasing temperatures in South Africa, while rainfall patterns remain highly erratic and unevenly distributed (Mackellar et al., 2014), wheat yields will continue to decline, thereby leading to food insecurity in the country as a whole.

The solution to this could be the improvement of wheat WUE, through selection of genotypes which are drought tolerant, and capable of producing high yields even under limited water availability. The residues of wheat could also be incorporated into soil after harvest to serve as an organic source of nutrients thus improving soil fertility status. This is of particular importance to farmers who cannot afford expensive mineral fertilizers, and also serves to reduce environmental damage caused by continued excessive use of chemical fertilizers.

Water use efficiency (WUE) is defined as the ratio of the total biomass or grain yield to water supplied (Sharma et al., 2005). Several researchers have looked at the strategies of improving WUE of wheat. These have mainly focused on finding stages of wheat growth that will improve WUE when irrigation water is applied (Shamsi et al., 2010; Zang and Oweis 1999; Ali et al., 2007). Zhang and Oweis (1999) for instance, reported that supplementary irrigation during booting to grain filling stage improves WUE of wheat when chances of rainfall are small. While Ali et al., (2007) found that irrigating wheat at early stages saved 68% of water compared with continuous irrigation. Other authors have focused more on the agricultural practises which can improve wheat WUE. Xie et al., (2005) found that plastic mulch increased WUE of wheat by 2-61% compared to the non-mulched treatment. While Fan et al., (2005) reported that the application of organic material (straw and manure) with fertilisers resulted in increased WUE of wheat and maize. Su et al., (2007) also reported that no till or subsoil tillage with mulching improved WUE of winter wheat. However, studies focusing on the selection of wheat genotypes exhibiting superior WUE under limited water supply, without the application of mulch or high fertilizer doses are lacking. Wheat has been identified as one of the crops with higher biomass allocation both to roots and shoots (Boogaard et al., 1996). It is envisaged that due to its high biomass production, wheat has potential for sequestering C hence can build up soil carbon stocks when planted or when residues are incorporated into the soil (Tahir et al., 2018). Not much is known however about the potential of its residues to sequester soil C or

improve fertility once incorporated into the soil. The aim of the present study was to assess water use efficiency and carbon sequestration potential of different wheat genotypes.

## **1.2 Specific objectives**

The study objectives were:

- (i) To identify the environmental factors affecting crop WUE through constructing a database on WUE of different crops at a global scale.
- (ii) To identify the most water use efficient wheat genotypes amongst the 100 wheat genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT).
- (iii) To assess soil carbon sequestration potential and mineralization patterns of residues from different selected wheat genotypes.

## **1.3 Hypotheses**

Based on the above objectives, the hypotheses for the study were:

- (i) WUE varies amongst different crop types and is significantly affected by climate.
- (ii) WUE of different wheat genotypes is higher under well-watered conditions compared to drought conditions.
- (iii) Selected wheat genotypes significantly differ in their C sequestration potential.
- (iv) Selected wheat genotypes exhibit different mineralization patterns.

## CHAPTER TWO

### ENVIRONMENTAL FACTORS AFFECTING CROP WATER USE

#### EFFICIENCY : A META-ANALYSIS

##### 2.1 Abstract

Water is becoming a limiting natural resource for agricultural production. The effects of rainfall pattern, soil type and climatic regime on soil water availability have been extensively investigated, but there is no consensus on the main factors affecting water use efficiency (WUE) of main crops, which was the motivation of the current study. The main factors controlling WUE in crops were evaluated using data from 546 experiments around the world published in ISI journal papers. The results showed that crop type had a significant effect ( $p < 0.05$ ) on WUE with grain crops producing on average 1.48 kg of dry grain per cubic meter ( $\text{m}^{-3}$ ) of water followed by legumes (0.80  $\text{kg m}^{-3}$ ), oilseeds (0.61  $\text{kg m}^{-3}$ ) and fibres crops (0.33  $\text{kg m}^{-3}$ ). Amongst cereals, maize (3.74  $\text{kg m}^{-3}$ ) followed by sorghum (3.34  $\text{kg m}^{-3}$ ) were more water use efficient crops than wheat (1.52  $\text{kg m}^{-3}$ ), barley (1.21  $\text{kg m}^{-3}$ ) and millet (0.47  $\text{kg m}^{-3}$ ). Overall, maize was the most water use efficient crop under optimal growing conditions, but sorghum was the most efficient under semi-arid and arid conditions with mean WUE of 1.5  $\text{kg m}^{-3}$  and 5.9  $\text{kg m}^{-3}$ , respectively. Summer crops showed higher WUE than winter crops due to differences in climatic conditions. WUE of crops increased from arid to tropical through sub-tropical climate. Moreover, WUE tended to positively correlate with soil organic carbon ( $r=0.77$ ) and clay content ( $r=0.20$ ), but negatively correlated with increasing soil bulk density ( $r=-0.25$ ). These results provide information that is important for making decisions on crop selection in a context of climate variability and for crop variety development with enhanced

WUE. However, there is need for more research for detailed understanding of the mechanisms responsible for the observed trends and causes of the high level of unexplained variability.

**Keywords:** climate variability, crop water use efficiency, crop management, photosynthetic prowess, soil water availability

## 2.1 Introduction

Water scarcity is a major global environmental problem of the 21<sup>st</sup> century (Srinivasan et al., 2012). Globally, agriculture accounts for 80–90% of all freshwater used by humans, and most of that is for crop production (Morison et al., 2008). While irrigation development has increased crop productivity in arid and semi-arid areas for decades, water scarcity and escalating costs of setting and managing the infrastructure hamper further expansion of irrigation in developing countries. Rising demand for water by other sectors such as domestic, mining, industries, the environment and recently, severe pressures from climate uncertainties, exacerbates water shortages for further irrigation development. Therefore, there is a need for new paradigms for agriculture to, at least, keep pace with rising demand for food.

Breeding efforts have focused on improving yield potential, drought tolerance and water use efficiency (WUE) of crops for years (Sivamani et al., 2000; Tilman et al., 2002; Condon et al., 2004; Blum, 2005; Ruggiero et al., 2017). Soil fertility and crop management techniques have also been developed to ensure better yields with less water (Evans and Sadler, 2008; Busari and Salako 2013; Busari et al., 2015). However, these strategies have not overcome the water scarcity issue. Growing selected crop types and promising varieties that are more efficient in the way they use available water could help in alleviating water scarcity.

Different disciplines define WUE differently. Originally, crop physiologists defined WUE as the amount of carbon assimilated and crop yield per unit of transpiration (Viets, 1962) and later it was referred to as amount of biomass or marketable yield per unit of evapotranspiration. Irrigation scientists view WUE as a ratio of total irrigation water transpired to water diverted from the source (Keller and Keller, 1995), while crop scientists define it as the ratio of total biomass or grain yield to water supplied (Sharma et al., 2015). However, the present paper



adopts the definition of WUE as the ratio of the total aboveground biomass achieved to the amount of water made available to the crop (i.e. stored in the soil plus rainfall and irrigation water). It is expressed as total aboveground biomass per unit of land area ( $Y$ ,  $\text{kg m}^{-2}$ ) divided by the amount of water consumed by the crop per unit land area ( $ET$ ,  $\text{m}^3 \text{m}^{-2}$ ), usually reported as mm of water needed to produce that yield (Blum 2005). WUE may also be influenced by the ability of soils to capture and store water, access the water stored in the soils and to convert that water into biomass.

While the impacts of water supply and climatic variability on soil water availability for crop production have been investigated extensively (e.g. Laporte et al., 2002; Kang et al., 2009; Chen et al., 2010; Iizumi and Ramankutty, 2015; Godde et al., 2016), there is still no clear consensus on the most important factors affecting WUE. Studies have reported a general increase of WUE with decreasing water supply (e.g. Eldem et al., 2001; Rusere et al., 2012; Mabhaudhi et al., 2013; Chibarada et al., 2015). Eldem et al. (2001) reported higher sunflower WUE under moisture stressed conditions than full irrigation and other treatments in a field study at Tekirdag, Turkey. Chibarada et al. (2015) reported the highest WUE in Bambara groundnut under severely moisture stressed conditions (25% FC, watering regime of  $0.006 \text{ g mm}^{-1}$ ) and lowest in non-stressed conditions (75% FC,  $0.003 \text{ g mm}^{-1}$ ) under a controlled experiment in South Africa. Rusere et al. (2012) also reported increasing winter silage maize WUE with decreasing amount of water applied in irrigation based trials in Mashonaland West Province of Zimbabwe. The type of irrigation system used also has a significant effect on WUE with drip system reported to have higher cotton WUE of  $0.77 \text{ kg m}^{-3}$  than the furrow system ( $0.49 \text{ kg m}^{-3}$ ) in Uzbekistan (Ibragmov et al., 2007). Regarding climate, Zhang et al. (2015) reported wheat, oats, potato and maize to have higher WUE in warm-dry than warm-wet environments of semi-arid northern China.

Other important factors that influence crop WUE include soil properties (Ismail and Ozawa, 2007; Dou et al., 2016), fertilizer usage (Fan et al., 2005), tillage (Su et al., 2007) and mulching (Xie et al., 2005). Ismail and Ozawa (2007) reported 45-64% higher WUE in crops grown on clayey than sandy soils. In a different study, Dou et al. (2016) also reported 25% higher WUE for rice grown on clayey than sandy loam soils. Fan et al. (2005) demonstrated the impact of fertilizer usage with fertilized wheat and maize having higher WUE of  $0.95 \text{ kg ha}^{-1} \text{ mm}^{-1}$  and  $0.96 \text{ kg ha}^{-1} \text{ mm}^{-1}$ , respectively, than unfertilized wheat ( $0.32 \text{ kg ha}^{-1} \text{ mm}^{-1}$ ) and maize ( $0.47 \text{ kg ha}^{-1} \text{ mm}^{-1}$ ) in Gansu, China. Conservation tillage resulted in superior crop WUE than conventional tillage practices in Henan Province, China (Su et al., 2007). In addition, mulched wheat had higher WUE ( $1.02 \text{ kg m}^{-3}$ ) than non-mulched wheat ( $0.875 \text{ kg m}^{-3}$ ) due to reduced losses of soil water through evaporation (Xie et al., 2005). WUE also vary with crop type with cereals tending to have higher WUE than pulses and oilseed crops (Norton and Waschmann, 2006; Sadras and McDonald 2012). Chibarabada et al. (2015) also showed that genotypic differences had significant effects on WUE, and they concluded that dark coloured Bambara nuts had higher WUE than light coloured genotypes.

Several studies have reported on crop WUE disparities with crop type, soil type, climate, amount of water applied and the growing season, making it difficult to compare WUE. However, data from such studies across the world provide an opportunity for comprehensive analysis seeking to draw general understanding on crop WUE. The data need integration over time, space and climate through focused analysis and interpretation for wider application. Therefore, the objectives of this paper were to integrate results from different studies worldwide in order to evaluate differences in WUE between main crops and to quantify the variations due to environmental conditions.

## **2.3 Methods and Materials**

### ***2.3.1 Database preparation***

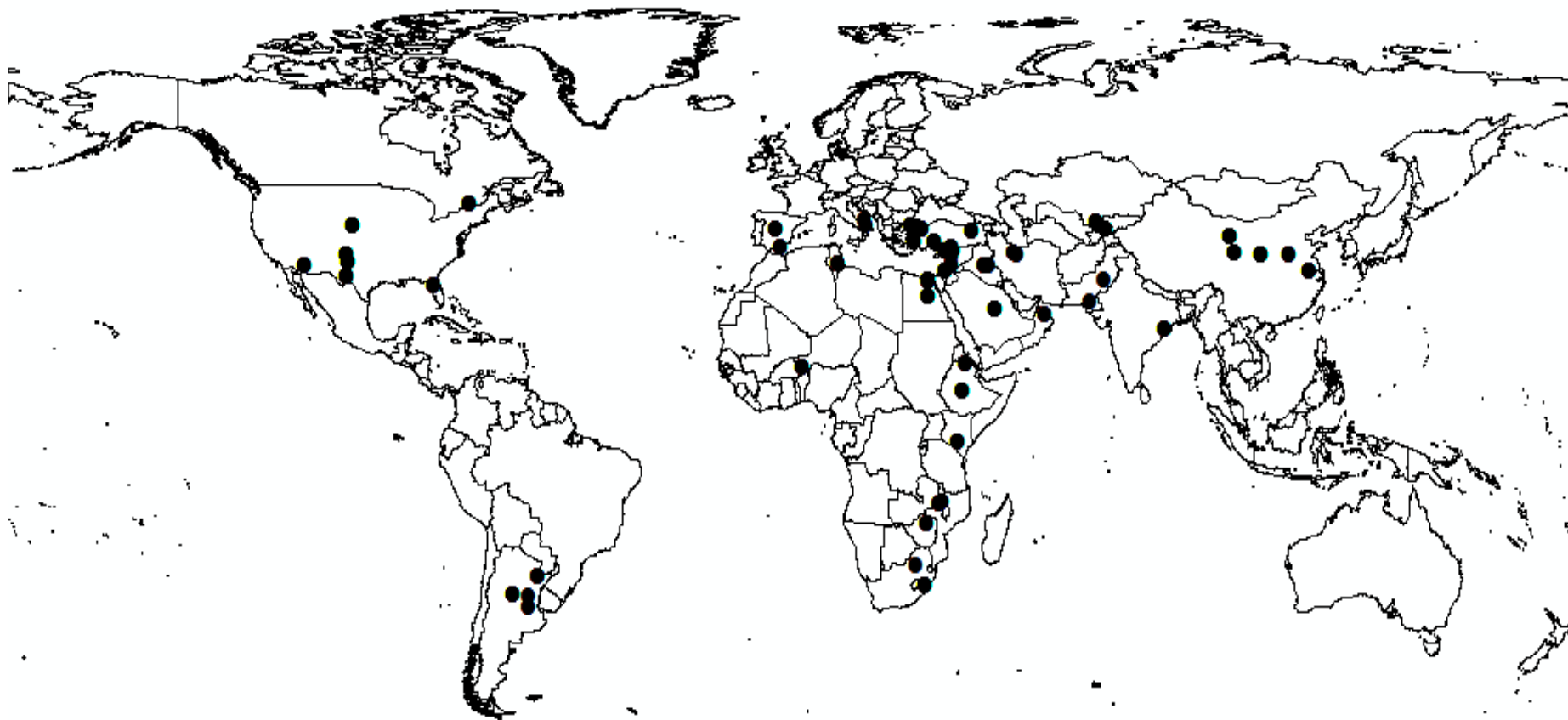
Online sources of published ISI journal papers such as Google Scholar, Science Direct, Springerlink, Scopus, Web of Science and Researchgate were searched for literature on WUE using key terms such as “crop water use”, “crop water use efficiency”, “water use efficiency” and “water use efficiency for different crops”. Only papers reporting on WUE for named field crops and environmental conditions, e.g. site, climate, soil type, whether the trial was in an open or controlled environment, amount of water applied and/or rainfall received were considered. In addition, data on plant population, fertilizer application rate, yield, and aboveground biomass were also captured. The final database, summarised in Table 2.1, consisted of 684 WUE data points from 60 peer-reviewed ISI journal papers. Figure 2.1 shows the locations of the data points. The main data contributors were USA (n=138), Turkey (n=130), China (n=100), Egypt (n=41) and Syria (n=40). Southern Africa had South Africa (n=16), Malawi (n=10) and Zimbabwe (n=6) as the main contributors.

**Table 2.1:** Summarized database compiled using data collected from ISI journal papers showing references (authors and years) and averages of selected environmental values (mean annual precipitation and temperature, MAP and MAT respectively; number of data points (n), total amount of water onto the plot, TW; available water capacity of soil, AWC%; topsoil bulk density, BD; topsoil clay, sand and silt content, Clay, Sand and Silt, respectively) and response variables (water use efficiency, WUE; and above ground biomass, Bio).

<b>Crop Name</b>	<b>Country</b>	<b>n</b>	<b>MAP</b> (mm y <sup>-1</sup> )	<b>MAT</b> (°C y <sup>-1</sup> )	<b>TW</b> (mm)	<b>AWC</b> %	<b>BD</b> (g cm <sup>-3</sup> )	<b>Clay</b> %	<b>Sand</b> %	<b>Silt</b> %	<b>WUE</b> (kg m <sup>-3</sup> )	<b>Bio</b> (t ha <sup>-1</sup> )	<b>References</b>
Wheat	Argentina	18	577	18	63						3.43	378	Abbate et al. 2004
Cowpea	Egypt	3	0	22	360	6	1.50				0.62		Aboamera, 2010
Maize	Egypt	6	0	21	935	25	1.31				1.20		Abuarab et al. 2013
Barley, Faba bean	Saudi- Arabia	16	100	16	479	8	1.58	3	81	16	0.72		Al-Neem 2008
Bambara nut	South Africa	12	845	19	110	34					4.79×10 <sup>-3</sup>	1	Chibarabada et al., 2015
Sorghum	USA	8	198	22	808						3.05		Conley et al., 2001
Sorghum	Spain	9	456	14	137	8		17	79	7	5.29	2856	Curt et al., 1995
Cotton, Maize	Turkey	20	657	18	374	21	1.46	12	45	26	1.32	1380	Dağdelen et al., 2006
Cotton	Turkey	4	657	18	548	23	1.46	17	55	28	0.85	968	Dağdelen et al., 2009
Sunflower	Turkey	15	575	14	387	15	1.56				0.74		Erdem et al., 2001
Elephant grass, Energycane ,Giantreed	USA	6	1228	27	1159						3.23	3270	Erickson et al., 2012
Maize, Wheat	China	6	580	14	766	16	1.35				2.36		Gao et al., 2009
Maize	USA	12	397	20	592	13					1.29	1626	Howell et al., 1995
Sorghum	Egypt	12	0	22	7300						5.99		Hussein and Alva, 2014
Cotton	Syria	8	120	17	600	19	1.16				0.66		Hussein et al., 2010
Cotton	Uzbekistan	9	228	26	541						0.67		Ibragimov et al., 2007
Maize	Iran	6	279	14	0	8	1.42				7.13		Kanani met al., 2016

Wheat	China	45	542	9	477		1.20	31	4	66	1.09	1036	Kang et al., 2001
Linseed,	India	9	1472	27	171	15	1.58	27	47	26	0.19	495	Kar et.al., 2007
Mustard,	Turkey	17	679	14	1391	15	1.40	33	24	44	3.45	2893	Kuscu and Demir, 2013
Safflower	Turkey	12	126	21	669	15					1.71		Kuscu et al., 2013
Maize	Turkey	12	409	6	474	8	1.42	26	37	38	1.13	502	Kuslu et al., 2010
Alfalfa	Egypt	20	0	22	1315						0.93		Mahmoud A.M. and Ahmed T.A. 2016
Sunflower	Italy	3	876	15	0	12		44	30	26	5.50	2694	Mastrorilli et al., 1999
Sorghum	South Africa	4	710	17	950						3.39	1318	Mengistu et al., 2016
Maize	Ethiopia	15	831	21	188	17	1.17				10.67	2115	Meskelu et al., 2014
Cotton	Pakistan	2	252	27	154						0.60		Muhammad et al.,2010
Millet	Tunisia	8	207	27	296						0.70	811	Nagaz et al., 2009
Cotton	Turkey	8	1109	18	1447	25	1.43	69	15	15	0.74		Onder et al., 2009
Chickpea	Syria	12	330	28	816						0.51	415	Oweis et al., 2004
Maize	Italy	3	650	15	340	18	1.25	40	6	53	1.93	1652	Paolo and Rinaldi 2008
Maize	USA	16	508	10	570	26					1.45	190	Payero, et al., 2008
Maize	USA	16	508	10	439	26					1.50	36	Payero et al., 2009
Sunflower	Pakistan	2	178	24	933						0.37		Qureshi et.al., 2005
Maize	Zimbabwe	6	850	22	388						11.81	4205	Rusere et al., 2012
Millet	Niger	8	252	29	0		1.65	4	91	5	0.24		Sivakumar and Salaam, 1999
Cotton	USA	42	142	25	501						0.22		Snowden et al., 2013
Maize,	USA	14	419	14	568						1.18		Stone et al., 1996
Sorghum,	China	4	614	14	439		1.38	15	2	83	1.18		Su et al., 2007
Sunflower	Turkey	22	318	12	365	14	1.59				1.18	1611	Tari,2016
Wheat	Malawi	10	1142	24	0			23	74	4	7.60		Teravest et al., 2015
Maize	China	12	540	7	345		1.30				0.09		Fan et al., 2005
Wheat,	USA	24	177	13	235						1.49		Tolk and Howell, 2003
Maize													
Sorghum													

Bean,											
Green gram	Uzbekistan	24	261	16	275	14		1.71	312	Webber et al., 2006	
Wheat	Syria	20	252	27	430	16		2.29	796	Zhang et al., 1998	
Wheat	China	24	129	7	433	23	1.37	0.95		Xie et al. 2005	



**Figure 2.1:** Global distribution of the sites where data used in the analysis were generated

### 2.3.2 Definitions of WUE and environmental factors

In the current study, WUE is defined as the amount of above ground biomass produced per unit volume of water used ( $\text{kg m}^{-3}$ ), regardless of differences in growing seasons for different crop types and varieties. Table 2.2 presents the definitions of selected soil and environmental conditions. The soil parameters used are topsoil clay, sand and silt content, bulk density, and amount of water at field capacity, permanent wilting point and available water capacity. The other environmental factors related to sites (LONG: longitude, LAT: latitude and Z: altitude), and climate (MAP: mean annual precipitation, and MAT: mean annual air temperature).

**Table 2.2:** Environmental factors, water use efficiency, yield and biomass used in the analysis

Environmental factors	Symbols	Units	Definitions
Mean annual precipitation	MAP	$\text{mm year}^{-1}$	Long-term (at least 30 year) mean precipitation per year for the study location from the papers
Mean annual air temperature	MAT	$^{\circ}\text{C year}^{-1}$	Long-term (at least 30 year) mean temperature per year for the study location from the papers
Longitude	LONG	$^{\circ}$	Longitude of the midpoint of study site as given in papers
Latitude	LAT	$^{\circ}$	Latitude of the midpoint of study site as given in papers
Altitude	Z	m.a.s.l	Average elevation above sea level of the study site as given in the papers
Rooting depth	Zd	m	Depth within the soil profile where most of the crop roots were found
Soil bulk density	BD	$\text{g cm}^{-3}$	Bulk density of the top soil layer as given in papers
Total Water	TW	mm	Total amount of water received by the crop during the full crop cycle (i.e. precipitation + irrigation)
Available water capacity	AWC	%	Amount of soil water available to crops (i.e. the calculated as the difference between field capacity and permanent wilting point)
Clay content	Clay	%	Average clay content (or fine textured soil particles) of the top soils in the plot
Silt content	Silt	%	Average silt content (or medium textured soil particles) of the top soils in the plot
Sand content	Sand	%	Average sand content (or coarse textured soil particles) of the top soils in the plot
Water use efficiency	WUE	$\text{kg m}^{-3}$	Amount of yield per unit volume of the amount of water received in the plot
Biomass	Bio	$\text{t ha}^{-1}$	Total biomass above the ground when the crop had matured



When climatic characteristics were not available in the papers, surrogate data for nearby prominent features (e.g. town) were used from Wikipedia. Environmental conditions varied widely and categories were generated for analyses (Table 2.3). Climate is defined here in terms of MAP and MAT following Mathew et al. (2017) and does not necessarily comply with Köppen (1936) system. Tropical represents hot ( $\text{MAT} > 20^\circ\text{C year}^{-1}$ ) and wet ( $\text{MAP} > 1000 \text{ mm year}^{-1}$ ) climate; subtropical depicts warm ( $\text{MAT}: 10\text{-}30^\circ\text{C year}^{-1}$ ) and arid to humid ( $\text{MAP}: 100\text{-}1110 \text{ mm year}^{-1}$ ); temperate represents cool ( $\text{MAT} < 10^\circ\text{C year}^{-1}$ ) and arid to moist ( $\text{MAP}: 120\text{-}1000 \text{ mm year}^{-1}$ ); while desert corresponds to warm ( $\text{MAT} > 15^\circ\text{C year}^{-1}$ ) and dry ( $\text{MAP}: 0\text{-}100 \text{ mm year}^{-1}$ ) zone. The transition from temperate to tropical climate represents increasing MAP and MAT. Soil texture (clay, sand and silt content) and amount of water supplied (precipitation plus irrigation) were categorised into low, medium and high class (Table 2.3). Crops were categorised by type and growing season (Table 2.3).

**Table 2.3:** Factors describing the environmental conditions and crop types used in the analysis

<b>Environmental factors</b>	<b>Remarks</b>	<b>Class range</b>	<b>Name</b>
Climate (MAP, mm year <sup>-1</sup> ; MAT, °C year <sup>-1</sup> )	Hot and wet	MAT > 20 MAP > 1000	Tropical
	Hot and dry	MAT > 15 MAP: 0-100	Desert
	Warm and arid-humid	MAT: 10-20 MAP: < 100-1110	Subtropical
	Cool and arid-moist	MAT < 10 MAP: 120-1000	Temperate
Clay (%)	Average clay content of the top soil horizon	0-20	Low
		20-35	Medium
		> 35	High
Sand (%)	Average sand content of the top soil horizon	0-25	Low
		25-50	Medium
		> 50	High
Silt (%)	Average silt content of the top soil horizon	0-20	Low
		20-40	Medium
		> 40	High

Total water (TW, mm)	Amount of water received by a plot (sums of precipitation and irrigation water applied)	0-500 500-1000 >1000	Low Medium High
Crop type	Field grains crops	wheat, maize, sorghum, millet, barley	Grain
	Field legume crops	Beans, peas	Legume
	Field oil seed crops	linseeds, mustard, safflower, sunflower, nuts	Oilseed
	Field fibre crops Field fodder crops	cotton alfalfa, energycane, giantreed, elephant grass	Fibre Grass
Growing season	Field crops grown under the different seasons	wheat, barley Maize, sorghum, pearl millet	Winter Summer

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Some of the factor classes were adapted from Mutema et al., (2015)

### 2.2.3 Data analyses

Descriptive statistics (minimum, maximum, median, mean, SEM: standard error of mean, quartile 1 and quartile 3 representing 25<sup>th</sup> and 75<sup>th</sup> percentiles respectively, skewness (Skew), kurtosis (Kurt) and coefficient of variation (CV%) were calculated for all study variables (Table 2.4). Mean WUE values were computed for different crop types, growing seasons and environmental factor classes. Significance differences between factor class values were tested at  $p < 0.05$  using t-test (Statistica 10.0). In addition, one to one Spearman rank correlations (Table 2.5) and principal component analysis (PCA) for multiple correlations (Figure 2.10) were performed. PCAs convert non-linear factors and variables into linear combinations called principal components (Jambu, 1991).

## 2.4 Results

### 2.4.1 General statistics of environmental variables and WUE

Table 2.4 shows that the type of environment and climatic conditions prevailing in a particular area varied widely, with MAP (mean  $420\pm 13$  mm  $y^{-1}$ ) ranging from 0 mm  $y^{-1}$  in Egypt where sorghum production was under sole irrigation (Hussein and Alva, 2014) to 1472 mm  $y^{-1}$  in India (Kar et al., 2007). The lowest irrigation was 12 mm applied to wheat in Turkey (Tari, 2016), while the highest amount was 7960 mm to sorghum in Egypt (Hussein and Alva, 2014) (Table 2.4 & 2.1). The average supplementary irrigation was  $569\pm 55$  mm. MAT (mean= $17.3\pm 0.3^{\circ}\text{C}$   $y^{-1}$ ) showed less variation than MAP (CV of 38 and 75% for MAT and MAP, respectively) and ranged from  $6.2^{\circ}\text{C}$   $y^{-1}$  in Turkey (Kuslu et al., 2010) to  $29^{\circ}\text{C}$   $y^{-1}$  in Niger (Sivakumar and Salaam, 1999) (Table 2.4 & 2.1). Relative humidity (RH,  $61.2\%\pm 0.5\%$ ) showed even lower variation (CV=11%) ranging from 48.4% in Syria (Hussein et al., 2010) to 81.5% in Argentina (Abbate et al., 2004). Bulk density of top soil (BD,  $1.39\pm 0.01$  g  $\text{cm}^{-3}$ ) ranged from 1.16 g  $\text{cm}^{-3}$  in Damascus, Syria (Hussein et al., 2010) to 1.65 g  $\text{cm}^{-3}$  in Niger (Sivakumar and Salaam, 1999). Top soil sand content ( $37.2\pm 2.4\%$ ) exhibited the highest variation amongst the textural properties (CV=82%) with a minimum value of 2.3% found in China (Su et al., 2007) and a maximum of 91% in Niger (Sivakumar and Salaam, 1999). Soil clay content in the top soil (mean= $24.6\pm 1.1\%$ ; CV=60%) varied from 3.24% in Saudi Arabia (Al-Neem, 2008) to 68.9% in Turkey (Onder et al., 2009) (Table 2.4 & 2.1). The lowest soil organic carbon content (SOCc) in the top soil was 0.2% for Niger (Sivakumar and Salaam, 1999) and the highest was 1.4% in Malawi (Teravest et al., 2015), with a mean of  $0.7\pm 0.1\%$ . Mean WUE was  $2.03\pm 0.11$  kg  $\text{m}^{-3}$  with values ranging from 0.003 kg  $\text{m}^{-3}$  for Bambaranuts in South Africa (Chibarabada et al., 2015) to 14.8 kg  $\text{m}^{-3}$  for maize in Ethiopia (Meskelu et al., 2014) (Table 2.4 & 2.1). Above-ground biomass (mean= $1191\pm 63$  g  $\text{m}^{-2}$ ) varied from 6.3 g  $\text{m}^{-2}$

for wheat in Argentina (Abbate et al., 2004) to 5950 g m<sup>-2</sup> for maize in Zimbabwe (Rusere et al., 2012). Plant population (107±32 plants m<sup>-2</sup>) varied widely (CV 199%), with maize, in China, being planted at 3.7 plants m<sup>-2</sup> (Gao et al., 2009), while alfalfa, in Turkey was densest at 1000 plant m<sup>-2</sup> (Kuscu et al., 2013). Fertilizer usage also varied with N application rate (145±6 kg ha<sup>-1</sup>) ranging from 36 kg ha<sup>-1</sup> in Egypt (Hussein and Alva, 2014) to 500 kg ha<sup>-1</sup> for barley in Saudi Arabia (Al-Neem, 2008) (Table 2.4 & Table 2.1).

**Table 2.4:** Descriptive statistics (Min: minimum, Max: maximum, Qt1 and Qt3: quartile 1 and quartile 3, respectively, SEM: standard error of mean, Skew: skewness, Kurt: kurtosis, CV%: coefficient of variation) of environmental factors and field crop variables (water use efficiency and biomass) included in the global database

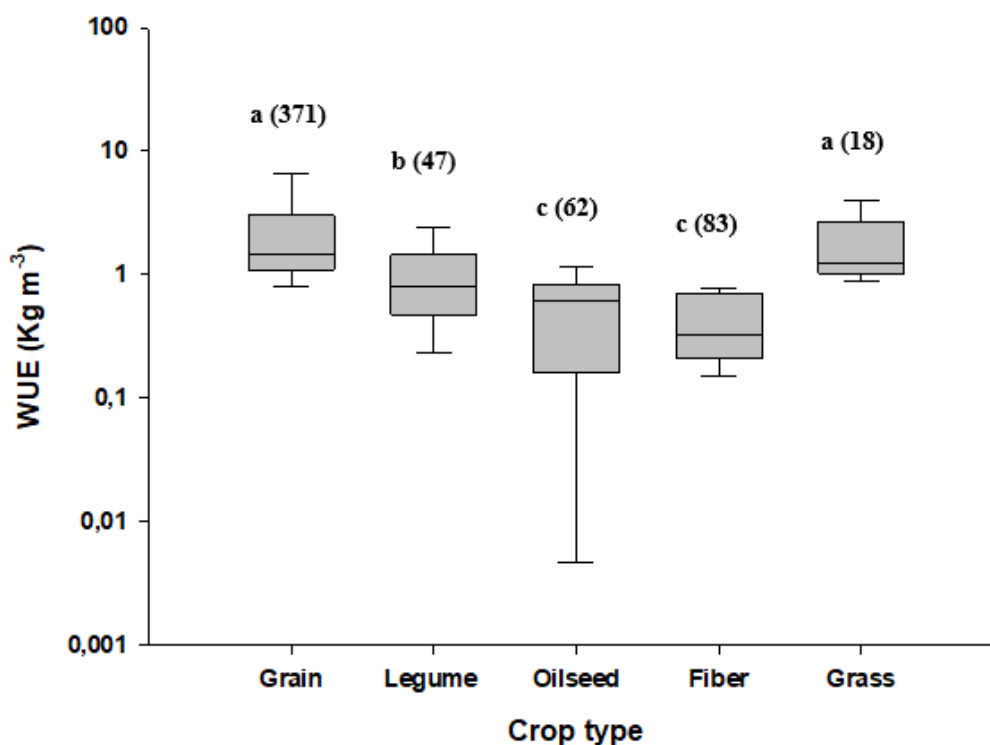
Variables	n	Mean	Median	Min	Max	Qt 1	Qt 3	SEM	Skew	Kurt	CV%
WUE	548	2.03	1.16	0.003	14.8	0.66	2.09	0.11	2.55	6.92	124
Biomass	301	1191	867	6.30	5950	395	1671	62.5	1.50	2.46	91
MAP	548	420	370	0.00	1472	142	575	13.4	1.17	1.72	75
MAT	548	17.3	17.5	6.20	29.0	11.9	21.9	0.28	0.04	-1.14	38
Z	548	686	608	4.00	1835	93.0	1146	22.7	0.27	-1.11	77
RH	174	61.2	59.5	48.4	81.5	56.8	64.4	0.50	0.62	0.33	11
BD	263	1.39	1.40	1.16	1.65	1.20	1.53	0.01	-0.04	-1.23	11
Clay	168	24.6	27.2	3.24	68.9	16.8	30.9	1.14	0.86	1.87	60
Sand	168	37.2	34.8	2.30	91.0	3.50	69.2	2.35	0.37	-1.29	82
Silt	168	36.3	28.0	3.40	82.5	15.6	65.6	1.78	0.24	-1.27	64
Soil pH	128	7.66	8.06	4.22	8.75	7.70	8.30	0.11	-1.78	1.98	16
SOM	158	1.70	1.55	0.34	5.50	0.53	2.15	0.10	1.53	2.49	72
SOCc	33	0.66	0.34	0.20	1.40	0.34	1.03	0.08	0.46	-1.30	67
SONc	120	0.17	0.11	0.00	1.25	0.10	0.11	0.03	3.16	8.43	181
Soil P	142	313	15.0	2.90	950	13.4	950	36.6	0.79	-1.39	139
Soil K	85	286	176	75.0	544	165	544	19.5	0.46	-1.51	63
Population	370	107	10.26	3.70	1000	7.14	42.1	11.1	2.80	8.04	199
Plot size	444	189	33.6	1.00	6400	21.0	225	29.6	9.04	87.7	330
Precipitation	425	252	240	0.00	1169	73.0	330	10.7	1.68	3.98	87
Irrigation	447	569	306	12	7960	150	518	55.1	5.28	28.3	205
Total water	548	660	445	0.00	7960	295	645	45.3	5.48	32.2	161
Water used	109	460	432	3.60	1304	272	593	28.5	0.95	1.06	65
ETc	268	530	472	37.9	1886	363	652	14.7	1.43	3.99	45
AWC%	306	16.7	16.0	6.32	26.0	12.5	22.5	0.32	0.17	-0.85	34
FC%	282	29.9	32.0	12.7	51.3	24.0	35.0	0.52	0.22	-0.05	29
WP%	264	13.4	11.2	6.00	26.3	9.00	17.5	0.34	0.71	-0.63	41
Zd	168	1.33	1.20	0.50	2.00	0.90	1.83	0.04	-0.06	-1.51	40
LAI	53	13.7	3.70	0.80	103	2.70	4.78	3.55	2.37	4.40	189
N rate	302	145	113	36	500	87.1	174	5.88	1.71	2.90	70
P rate	270	81.2	60.0	8.40	400	31.00	100	4.66	2.46	7.04	94
K rate	86	109	75.0	20.0	840	60.0	100	17.9	4.07	15.8	152

MAP=mean annual precipitation (mm yr<sup>-1</sup>); MAT=mean annual air (°C); Z=altitude (masl); RH%=relative humidity (%); BD=top soil bulk density (g cm<sup>-3</sup>); Clay, Sand and Silt for top soil clay, sand and silt content (%); Population=plant population (plants m<sup>-2</sup>); Irrigation=amount of irrigation water applied (mm); Total water= Sum of precipitation and irrigation (mm), Water used= amount of water used by crop (mm); ETc=Crop evapotranspiration for the entire crop cycle (mm); AWC%=available water capacity of soils (%); FC=field capacity (%), WP=permanent wilting point (%); Zd=rooting depth of crops (m); LAI=leaf area index; N, P and K

rate=application rate of N, P and K ( $\text{kg ha}^{-1}$ ); Biomass=above ground biomass ( $\text{g m}^{-2}$ ); WUE=water use efficiency ( $\text{kg m}^{-3}$ )

### 2.4.2 Impact of crop type on WUE

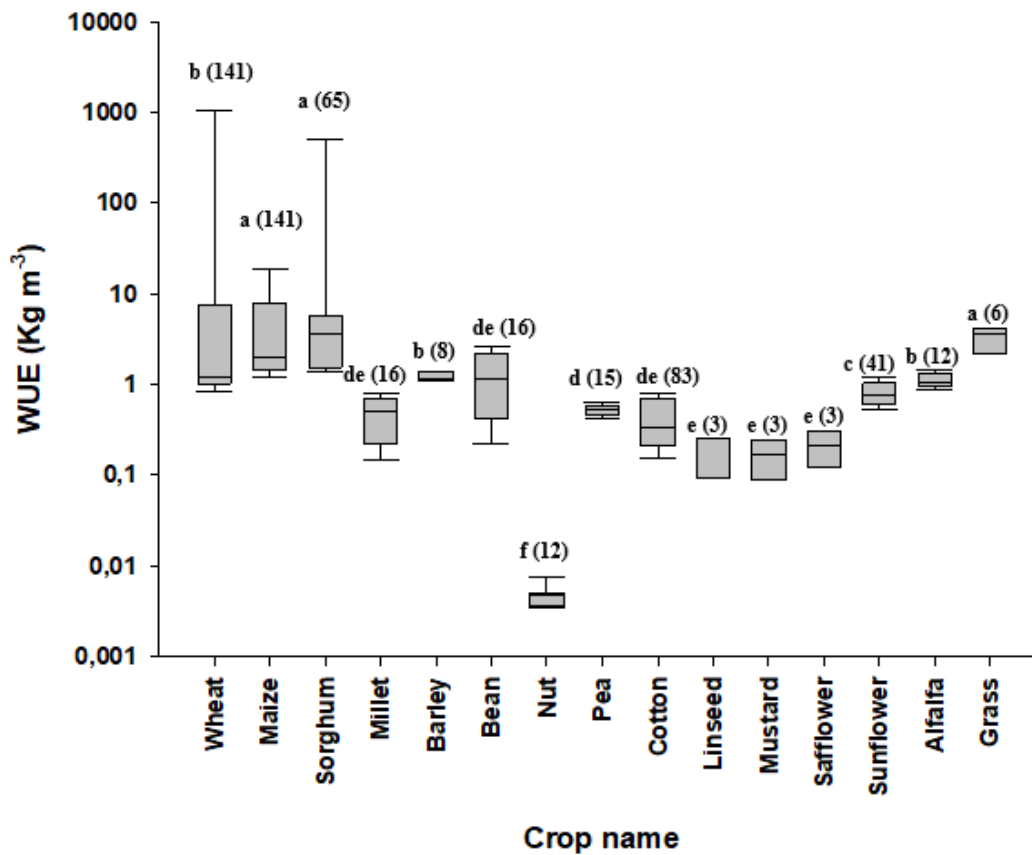
Grain crops had the highest WUE amongst the cultivated crop types studied (Figure 2.2). Their median WUE ( $1.48 \text{ kg m}^{-3}$ ) was not significantly different from non-cultivated grasses ( $1.24 \text{ kg m}^{-3}$ ). WUE decreased from grain to legume ( $0.8 \text{ kg m}^{-3}$ ), oilseed ( $0.61 \text{ kg m}^{-3}$ ) and to fibre crops ( $0.33 \text{ kg m}^{-3}$ ), with all differences significant except between oilseed and fibre crops.



**Figure 2.2:** Box plots comparing WUE ( $\text{kg m}^{-3}$ ) variations among field crop types/categories used in the analysis. Box plots accompanied by similar letters were not significantly different at  $<0.05$ . Numbers between brackets are the sample sizes. Y-axis is in logarithmic scale. See Table 2.3 for the crop type classes

Natural grass (median= $3.6 \text{ kg m}^{-3}$ ) had 1.5 and 2 fold greater WUE than sorghum and maize, respectively (Figure 2.3). Maize and sorghum WUE were significantly higher than barley ( $1.17 \text{ kg m}^{-3}$ ) and wheat ( $1.15 \text{ kg m}^{-3}$ ). Pearl millet had the lowest WUE ( $0.51 \text{ kg m}^{-3}$ ) amongst the

grains crops. Beans ( $1.15 \text{ kg m}^{-3}$ ) and alfalfa ( $1.07 \text{ kg m}^{-3}$ ) had similar WUE to wheat. However, both had significantly higher WUE than sunflower ( $0.77 \text{ kg m}^{-3}$ ) (Figure 2.3). In turn, sunflower had significantly higher WUE than peas ( $0.52 \text{ kg m}^{-3}$ ), cotton ( $0.33 \text{ kg m}^{-3}$ ), linseeds ( $0.25 \text{ kg m}^{-3}$ ), safflower ( $0.21 \text{ kg m}^{-3}$ ) and mustard ( $0.17 \text{ kg m}^{-3}$ ). Overall, Bambaranuts had the lowest WUE of  $0.003 \text{ kg m}^{-3}$  (Chibarabada et al., 2015) (Figure 2.3).

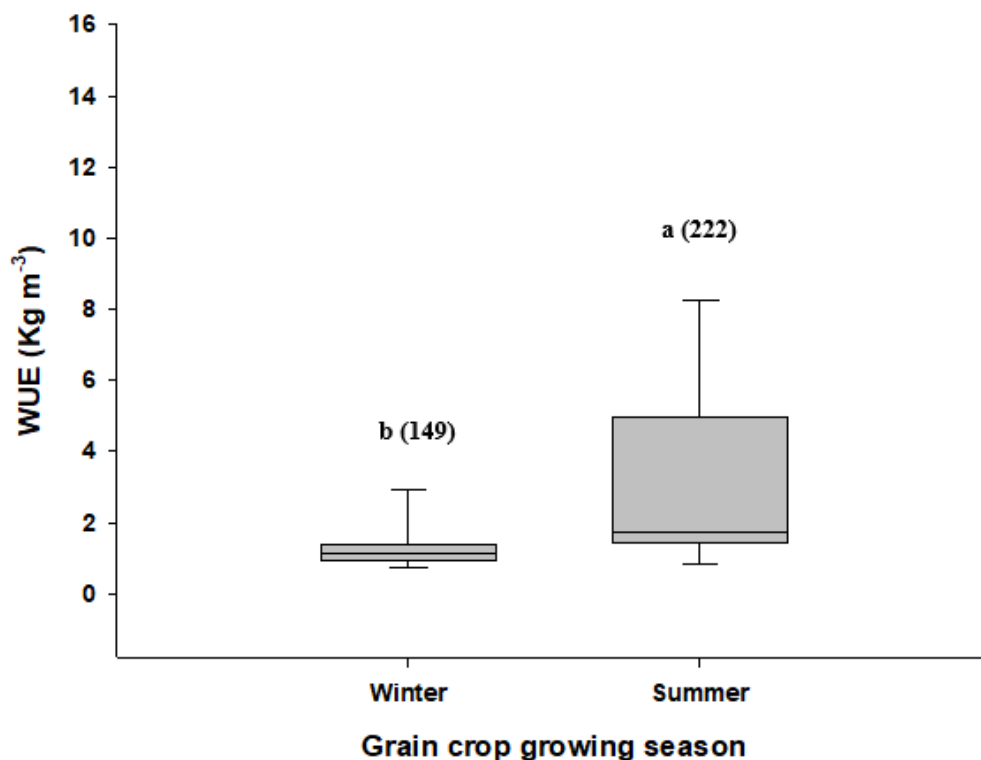


**Figure 2.3:** Box plots comparing WUE ( $\text{kg m}^{-3}$ ) variations among the different field crops used in the analysis. Box plots accompanied by similar letters were not significantly different at  $<0.05$ . Numbers between brackets are the sample sizes. Y-axis is in logarithmic scale.

### 2.4.3 Impact of environmental conditions on WUE

#### 2.4.3.1 Climate

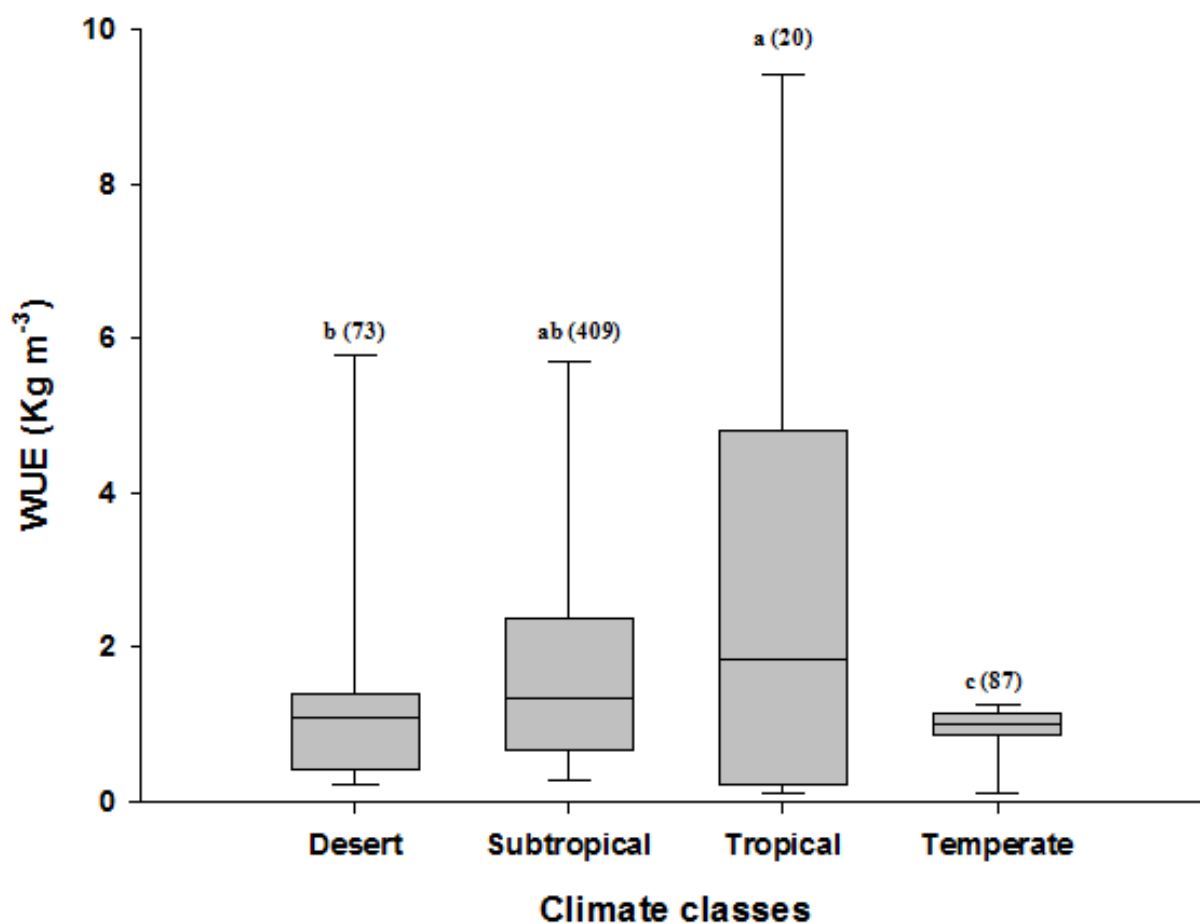
Growing season showed significant effect on WUE with summer crops having higher WUE ( $1.72 \text{ kg m}^{-3}$ ) than winter crops ( $1.15 \text{ kg m}^{-3}$ ) suggesting that growing crops off-season might decrease WUE by over 30% (Figure 2.4).



**Figure 2.4:** Box plots comparing WUE ( $\text{kg m}^{-3}$ ) variations for field grain crops grown in winter and summer used in the analysis. Box plots accompanied by similar letters were not significantly different at  $p < 0.05$ . Numbers between brackets are the sample sizes. See Table 2.3 for the crops in each season class

Figure 2.5 confirms that WUE increase with MAP regime from desert (median  $1.08 \text{ kg m}^{-3}$ ) to subtropical ( $1.34 \text{ kg m}^{-3}$ ) and to tropical climate ( $1.83 \text{ kg m}^{-3}$ ). The increase from desert to subtropical climate was 24%, while that from subtropical to tropical climate was 37%.

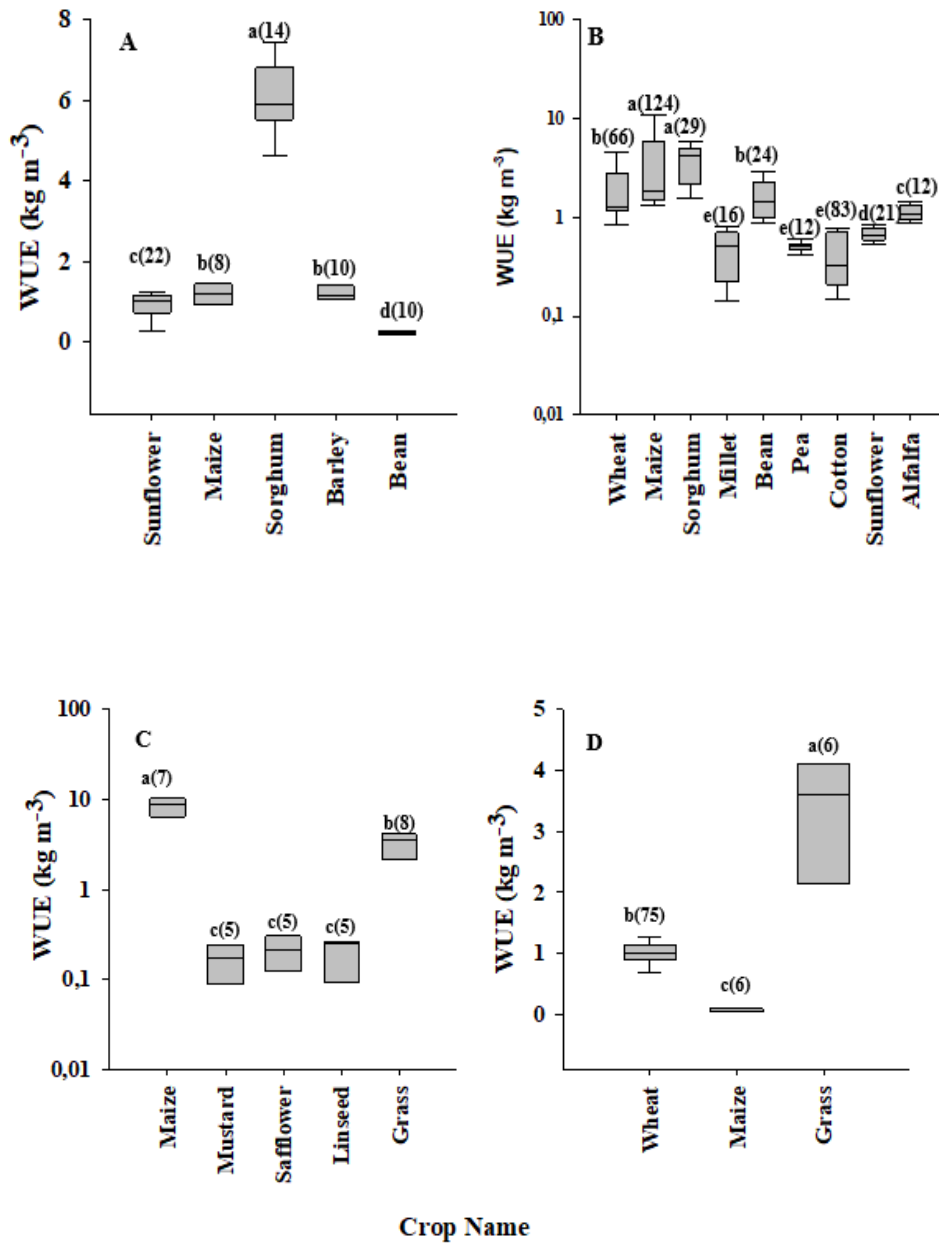




**Figure 2.5:** Box plots comparing WUE ( $\text{kg m}^{-3}$ ) variations for field crops under different climatic regions. Box plots accompanied by similar letters were not significantly different at  $p < 0.05$ . Numbers in brackets are the sample sizes. See Table 2.3 for climate class

Sorghum had the highest WUE ( $5.6 \text{ kg m}^{-3}$ ) followed by maize ( $1.21 \text{ kg m}^{-3}$ ) and barley ( $1.2 \text{ kg m}^{-3}$ ) under desert conditions (Figure 2.6A). However, maize WUE ( $3.92 \text{ kg m}^{-3}$ ) was not significantly different from sorghum ( $3.78 \text{ kg m}^{-3}$ ) under subtropical conditions (Figure 2.6B). Surprisingly, wheat and beans were also not significantly different under the subtropical conditions, but peas ( $0.51 \text{ kg m}^{-3}$ ), pearl millet ( $0.47 \text{ kg m}^{-3}$ ) and cotton ( $0.45 \text{ kg m}^{-3}$ ) showed significantly lower WUE. Maize had significantly higher WUE ( $8.37 \text{ kg m}^{-3}$ ) than grass ( $3.37 \text{ kg m}^{-3}$ ) in the tropical climate (Figure 2.6C). However, grass WUE was still higher than mustard ( $0.71 \text{ kg m}^{-3}$ ), safflower ( $0.21 \text{ kg m}^{-3}$ ) and linseed ( $0.20 \text{ kg m}^{-3}$ ). Temperate climate

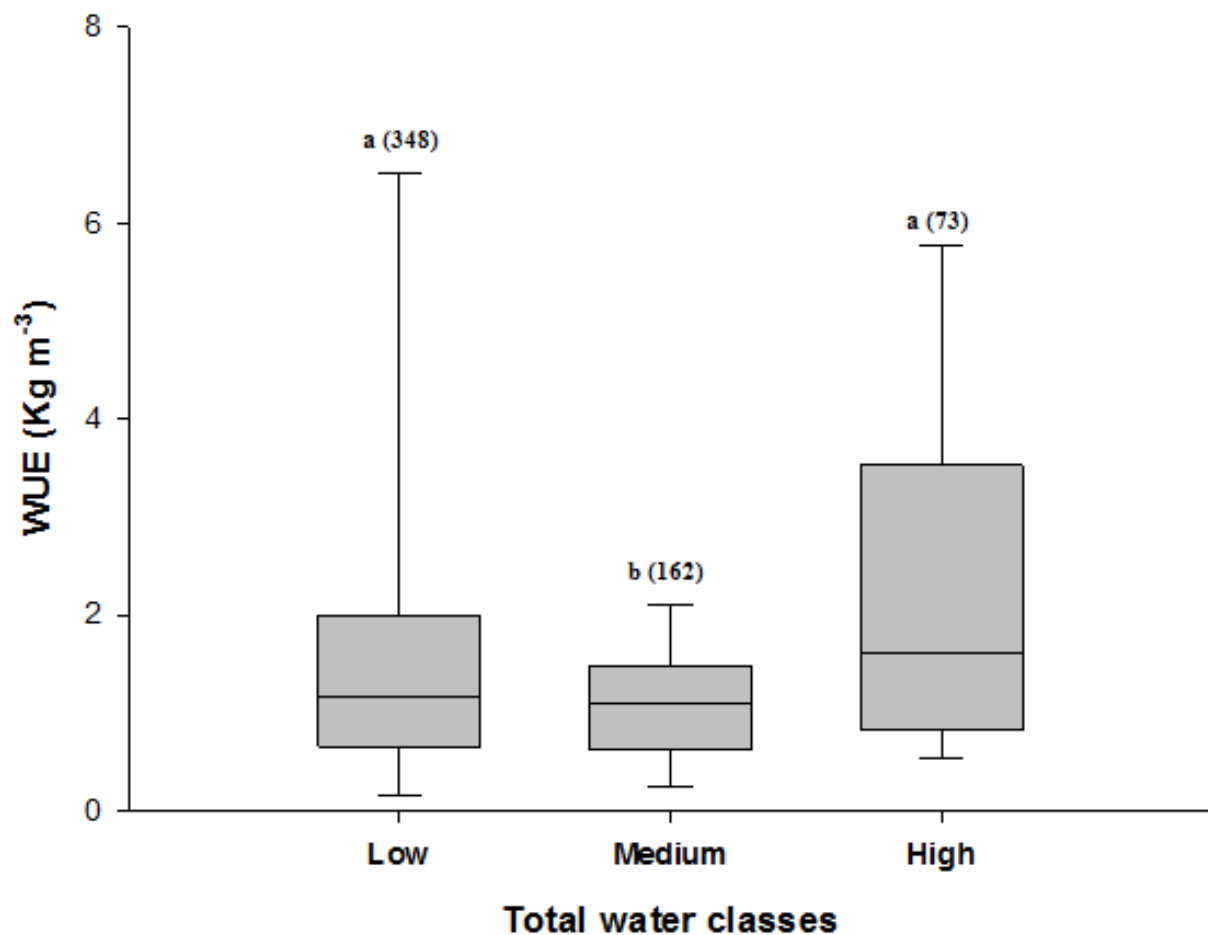
had fewer crop types than other climates (Figure 2.6D), and grass had highest WUE (3.23 kg m<sup>-3</sup>) followed by wheat (0.97 kg m<sup>-3</sup>) and lowest was maize (0.9 kg m<sup>-3</sup>).



**Figure 2.6:** Box plots comparing WUE (kg m<sup>-3</sup>) variations amongst field crops grown in different environments (A) Desert, (B) Subtropical (C) Tropical and (D) Temperate. Box plots accompanied by similar letters were not significantly different at p<0.05. Numbers in brackets are the sample sizes.

### 2.4.3.2 Water received

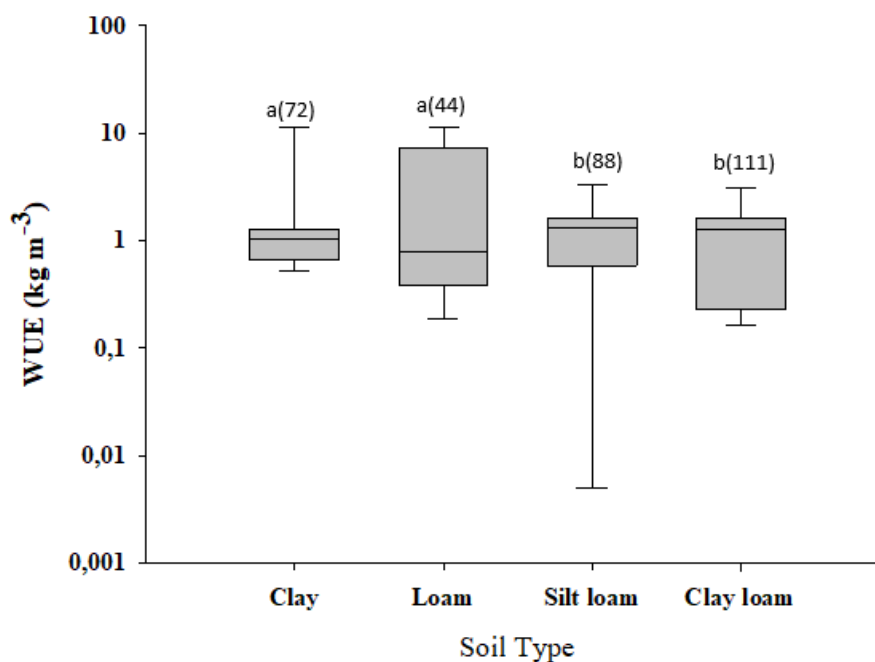
The amount of precipitation had significant effect on WUE ( $r_s=0.16$ ) and subsequently, total water (precipitation plus irrigation) received also showed significant effect on WUE ( $r_s=0.21$ ) (Table 2.5). WUE decreased significantly from a lowly watered regime (median=1.17 kg m<sup>-3</sup>) to a moderately watered regime (1.08 kg m<sup>-3</sup>) (Figure 2.7). Unexpectedly, a fully watered regime had the highest WUE (1.60 kg m<sup>-3</sup>); however, it was not significantly different from the regime receiving lower amount of water. The fully watered regime resulted in 37 and 48% higher WUE than lower and moderately watered regimes, respectively.



**Figure 2.7:** Box plots comparing WUE (kg m<sup>-3</sup>) variations for different field crops under three different total water classes. Box plots accompanied by similar letters were not significantly different at  $p<0.05$ . Numbers in brackets are the sample sizes. See Table 2.3 for total water class definitions.

### 2.4.3.3 Soil texture

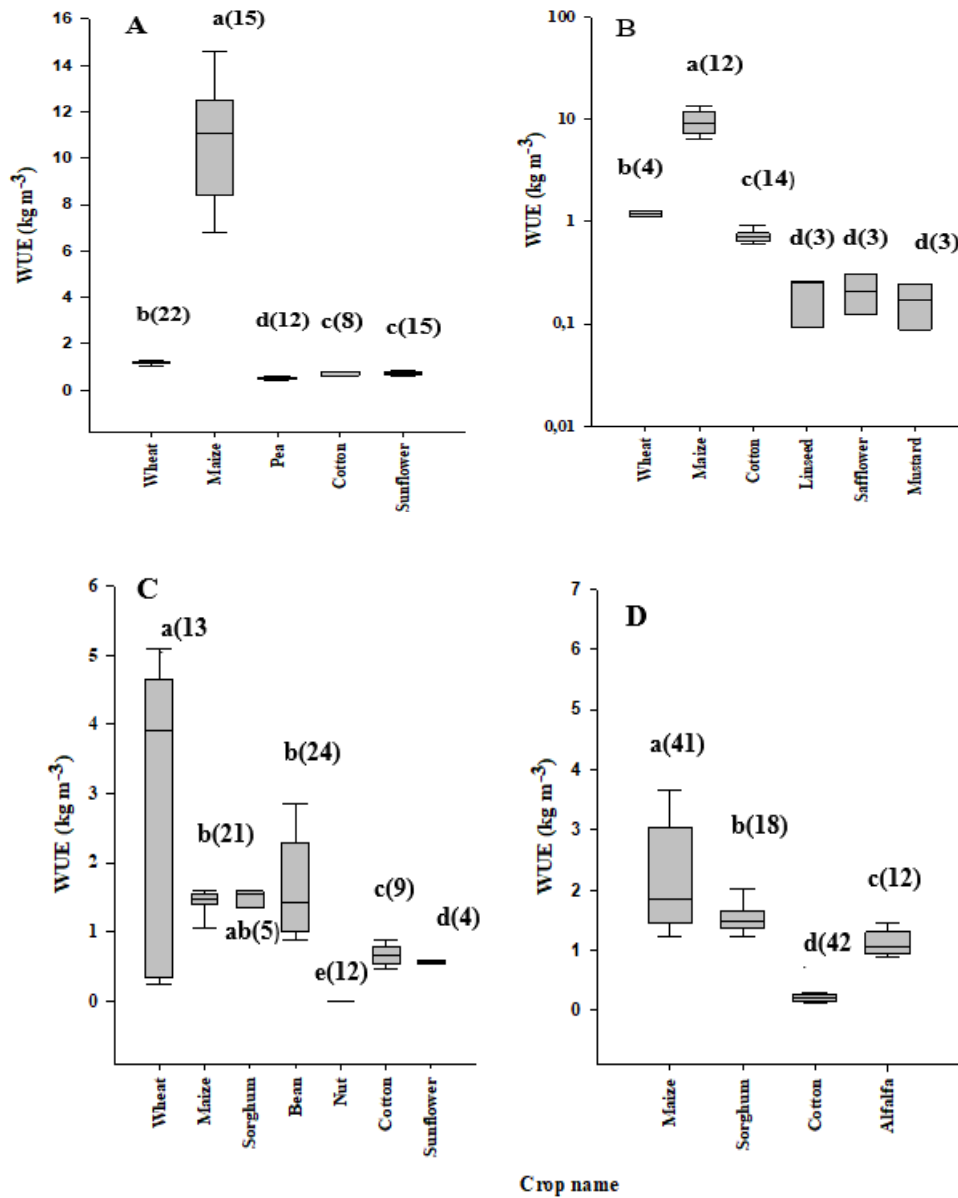
Clay content was the only textural property that exhibited a significant effect on WUE ( $r=0.20$ ) (Table 2.5). The other soil physical properties exhibited moderate effects with, for example, bulk density showing negative effect on WUE ( $r=-0.25$ ). Box plots showed a significant effect of soil texture on WUE (Figure 2.8). WUE of field crops in general was higher under silt loam textured soil ( $3.47 \text{ kg m}^{-3}$ ). However non-significant differences were observed between silt loam and clay loam soil ( $2.9 \text{ kg m}^{-3}$ ). WUE of field crops was significantly lower in both clay ( $1.43 \text{ kg m}^{-3}$ ) and loam soil ( $1.27 \text{ kg m}^{-3}$ ) (Figure 2.8).



**Figure 2.8:** Box plots comparing field crops WUE ( $\text{kg m}^{-3}$ ) variations under different soil types. Box plots accompanied by similar letters were not significantly different at  $p < 0.05$ . Numbers in brackets are the sample sizes. Y-axis in logarithmic scale.

Figure 2.9 indicates that maize was the most water use efficient crop under all soil types except for silt loam soil, as its WUE on clay was ( $10.67 \text{ kg m}^{-3}$ , Figure 2.9A), loam ( $9.47 \text{ kg m}^{-3}$ ,

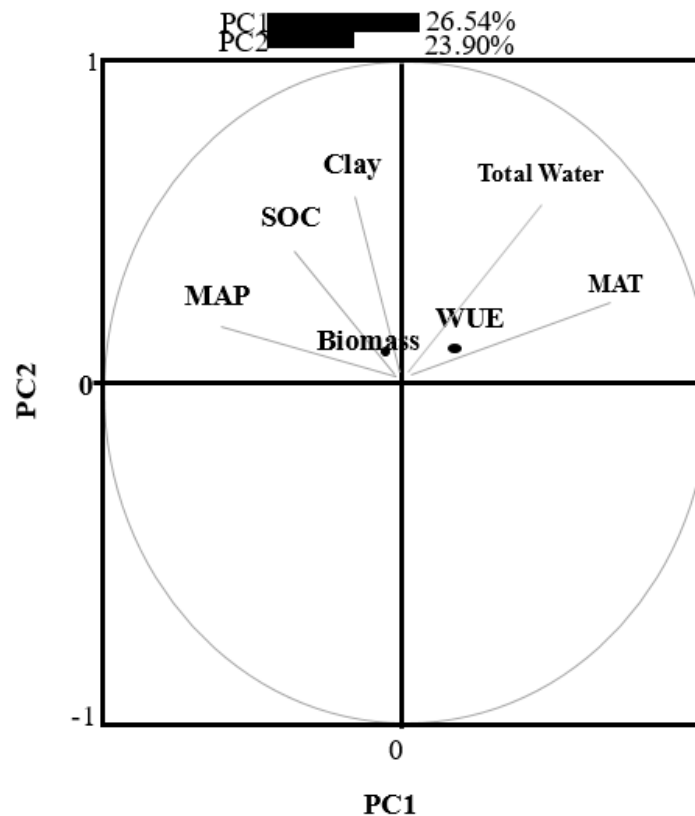
Figure 2.9B) and clay loam soils ( $2.31 \text{ kg m}^{-3}$ , Figure 2.9D). Safflower ( $0.21 \text{ kg m}^{-3}$ ), linseed ( $0.2 \text{ kg m}^{-3}$ ) and mustard ( $0.18 \text{ kg m}^{-3}$ ) had much lower WUE than cotton on loam soil. Wheat had the second highest WUE on clay ( $1.18 \text{ kg m}^{-3}$ ) and loam soil ( $1.18 \text{ kg m}^{-3}$ ); but it had the highest WUE on silt loam soil ( $3.04 \text{ kg m}^{-3}$ , Figure 9C). Sorghum ( $1.48 \text{ kg m}^{-3}$ ) and maize ( $1.43 \text{ kg m}^{-3}$ ) were not significantly different in WUE on silt loam soil; while cotton had the lowest WUE ( $0.28 \text{ kg m}^{-3}$ ) on clay loam soil (Figure 2.9D).



**Figure 2.9:** Box plots comparing WUE ( $\text{kg m}^{-3}$ ) variations for field crops under different soil types (A) Clay soil, (B) Loamy soil, (C) Silt loam soil and, (D) Clay loam soil. Box plots accompanied by similar letters were not significantly different at  $p < 0.05$ . Numbers in brackets are the sample sizes.

#### 2.4.4 Principal component and correlation analysis of environmental factors affecting WUE

Five principal components accounted for 50.44% of the total variation, with the first two principal components PC1 and PC2 accounting for 26.54 and 23.9 %, respectively (Figure 2.10). PC1 was closely associated with total water and MAT on the positive coordinates. On the other hand, PC2 was closely associated with clay and MAP with both factors exhibiting positive coordinates. This PC represented the axis of increasing soil moisture. Therefore, the results of this PC analysis implied that WUE increased with soil moisture and temperature. Soil clay content and SOC were also important promoters of higher WUE. These associations are in general agreement with the Spearman rank correlation results in Table 2.5.



**Figure 2.10:** Principal component analysis of selected environmental factors and the water use efficiency parameters (Biomass and WUE). Environmental factors were used as variables for analysis while the water use efficiency parameters were the supplementary variables.

Although many factors were studied and had influence on crop WUE, only a few factors had positive correlations with WUE (Table 2.5). These were observed between WUE and MAP ( $r=0.27$ ,  $p<0.05$ ), MAT ( $r= 0.09$ ,  $p<0.05$ ), Clay content ( $r=0.20$ ,  $p<0.05$ ), RH ( $r=0.45$ ,  $p<0.05$ ), SOCc ( $r=0.77$ ,  $p<0.05$ ), OM ( $r= 0.17$ ,  $p<0.05$ ) and Soil P ( $r= 0.45$ ,  $p<0.05$ ). Positive correlation were also observed between WUE and Soil K ( $r= 0.63$ ,  $p<0.05$ ), crop type ( $r=0.23$ ,  $p<0.05$ ), precipitation ( $r=0.16$ ,  $p<0.05$ ), total water ( $r=0.21$ ,  $p<0.05$ ), AWC ( $r=0.16$ ,  $p<0.05$ ), Zd ( $r=0.25$ ,  $p<0.05$ ), LAI ( $r=0.17$ ,  $p<0.05$ ) and K application rate ( $r=0.14$ ,  $p<0.05$ ). Negative correlations were however observed between WUE and climate ( $r=-0.14$ ,  $p<0.05$ ), BD ( $r=-0.25$ ,  $p<0.05$ ), SONc ( $r=-0.10$ ,  $p<0.05$ ), plant population ( $r=-0.22$ ,  $p<0.05$ ) and water used ( $r=-0.06$ ,  $p<0.05$ ) (Table 2.5).



**Table 2.5:** Spearman rank correlations between selected controlling factors and WUE

<b>Variables</b>	<b>WUE</b>
MAP	0.27*
MAT	0.09*
Altitude	0.00
Climate	-0.14*
RH%	0.45*
BD	-0.25*
Clay	0.20*
Sand	-0.11
Silt	-0.01
Soil type	0.49
Soil pH	-0.04
OM	0.17*
SOCc	0.77*
SONc	-0.10*
Soil P	0.45*
Soil K	0.63*
Crop type	0.23*
Plant population	-0.22*
Plot size	0.04
Precipitation	0.16*
Irrigation	0.02
Total water	0.21*
Water used	-0.06
AWC%	0.16*
Zd	0.25*
LAI	0.17*
ETc	-0.09
N application rate	0.04
P application rate	0.03
K application rate	0.14*

\* significant at  $p < 0.05$  MAP=mean annual precipitation; MAT=mean annual air temperature; RH%=relative humidity of the atmosphere; OM=soil organic matter content; SOCc=soil organic carbon content; SONc=soil organic nitrogen content; Soil P=soil phosphorus content; Soil K=soil potassium content; Total water=some of rainfall and irrigation water applied to a crop; AWC%=available water capacity of the soil; FC%=field capacity; WP%=permanent wilting point; LAI=leaf area index; ETc=evapotranspiration of the crop, Precipitation=amount of rainfall and snow received during the growing season.

## **2.5 Discussion**

### ***2.5.1 Impact of crop type on WUE***

Water use efficiency (WUE) varied with crop type, with our results indicating that maize and sorghum tended to have higher WUE than other members of the grass family such as wheat (Figure 2.3). The significant differences in WUE between maize and sorghum on one hand and wheat on the other hand may be explained by differences in their photosynthetic pathway. These two groups have different photosynthetic pathways linked to the way they evolved genetically. Photosynthetic pathways have been used to classify plants into C4 and C3 types. C4 plants (which include sugarcane, maize, sorghum and millet) originated in subtropical areas, while C3 plants (which include wheat, barley and rice) populated a far broader range of climates (Yamori et al., 2014). Studies have generally shown that C4 plants tend to be more water use efficient than C3 species under both natural and managed ecosystems (Blenkenagel et al., 2018; Way et al., 2014; Zwart and Bastiaanssen, 2004). The higher WUE in C4 than C3 crops is attributed to increased CO<sub>2</sub> concentration mechanisms in the special bundle sheath cells of C4 plants, which allow higher photosynthetic rates while reducing stomatal conductance. The reduced stomatal conductance reduces transpiration, resulting in water savings (Sage and Manson, 1999). However, whether C4 are more water use efficient than C3 plants or not appears to be controlled by the growing environment; for example, C4 plants tend to outperform C3 plants in photosynthetic prowess under hot and dry conditions, while C3 plants perform better under sufficient water and sunlight conditions.

The growing conditions used in the current analysis appear to have favoured C4 plants more than C3 plants. These findings agree with results from other studies. Katerji and Mastorilli (2014), in a Mediterranean environment found higher WUE in sorghum than other major grain crops including wheat and barley. The impact of growing environment may also explain the

low WUE for pearl millet, a C<sub>4</sub> plant type, which was even lower than wheat WUE. Pearl millet is mostly grown in marginal environments of arid and semi-arid regions of Africa and Asia (Vadez et al., 2012), where research efforts are generally limited. Hence, research related activities around this crop tend to occur in environments less favourable for its optimum performance.

The current results also showed that grain crops had the highest WUE (Figure 2.2), which was largely anticipated because the group was dominated by C<sub>4</sub> crops. Thus, WUE tended to decrease from grain crops to legumes, oilseeds and to fibre crops, which was also expected because other studies elsewhere showed a similar trend. Angadi et al. (2008), whilst evaluating WUE of different crop types, found that wheat (grain crop) had the highest WUE followed by pulses (legumes) with the oilseeds having the lowest WUE. Legumes are C<sub>3</sub> plants and would be expected to have lower WUE than grain crops dominated by C<sub>4</sub> plants. It is also generally known that yield per unit area basis tends to decrease as crops are improved for such properties as high protein, oil and fibre production, and this might explain the general decrease because WUE was based on yield. On the basis of this, the very low WUE for nuts (Figure 2.3) would not be surprising because they are generally improved for protein and oil production. Another possible explanation for the low nut WUE could be errors when normalising young crop WUE data to full maturity basis. WUE data for nuts mostly came from young plants in the early stages of their growth cycles. The lack of significant difference between oilseeds and fibre crops may be attributed to higher variability of WUE under oilseeds.

The results also showed that summer grain crops had significantly higher WUE than winter grain crops (Figure 2.4) and that was reflective of the performance of C<sub>4</sub> vs. C<sub>3</sub> plants. The summer crop group consisted of maize, sorghum and millet which are C<sub>4</sub> types, while the winter group was composed of wheat and barley which are C<sub>3</sub>. Summer conditions might also be more conducive for crop productivity especially in the southern hemisphere where summers

are characterised by relatively higher temperatures, soil moisture and more sunshine hours. Southern hemisphere winters are generally cooler and drier with less sunshine hours. However, high variability of WUE for both groups might be indicative of the existence of other critical controls of WUE. Thus, the impacts of selected environmental controls were also elucidated.

## ***2.5.2 Impact of environmental conditions on WUE***

### ***2.5.2.1 Climate***

Climate is very crucial for growth, it largely determines whether crops would be successful or not in a particular environment. Climatic factors (MAP and MAT) showed significant effects on WUE (Table 2.5), with a tendency for WUE to increase from desert-like environments to subtropical and to tropical zones (Figure 2.5). There was no significant difference between WUE in tropical and subtropical environments. This is mainly due to the greater number of crops favoured by these environments compared to desert and temperate environment (Figure 2.6). Maize and sorghum exhibited higher WUE in desert, subtropical and tropical environments (Figure 2.6A-6C). This was because both maize and sorghum are classified as C4 crops. They both have higher drought tolerance and WUE compared to other crops (i.e. wheat, cotton, sunflower, barley, millet) (Chipanshi et al., 2003). The increase of WUE from desert to subtropical and tropical environments can be explained in terms of high temperature which decrease with the change of environmental conditions. Heat stress on crops is likely to be highest in the deserts due to a combination of high temperatures and low humidity (Sherwood and Huber, 2010; Tardieu, 2013). This also drives soil moisture losses through evapotranspiration. The combined effect of these two factors depresses crop WUE (Shah and Paulsen, 2003; Boutraa et al., 2010; Tardieu, 2013) due to subdued yields. Heat and soil moisture stresses generally decrease with transition towards the tropical region due to a general increase of precipitation which raises humidity levels. Though subtropical and tropical regions

may be characterised by high average air temperatures, higher precipitation incidences (especially in the tropics) tend to increase soil water availability and air humidity which results in a number of crops being favoured by these environments (Figure 2.6). This school of thought is supported by the PCA results (Figure 2.10) that showed positive WUE with MAT. It was also clear that WUE tended to increase with precipitation (MAP), total irrigation applied (Total Water), soil clay (Clay) and carbon content (SOCc). The temperate climate produced field crops that had the least variation in WUE (CV 42%), which was much lower than for the other climates (119, 120 and 114% for desert, subtropical and tropical climate, respectively). Low WUE in temperate climate (Figure 2.5) was also confirmed by fewer crops found under this environment (Figure 2.6). Despite relatively high soil moisture levels due to low air temperatures in the temperate regions, most crop growth cycles are quite long due to low metabolic rates (Hendrickson et al., 2004), yet yields seldom outperform warmer regions by significant margins. Since WUE used in the current study was based on yield, it therefore means that WUE is bound to be much lower in temperate than warmer regions where plant growth is faster. Of course, the foregoing assumes good management practices for optimal production in all regions. It is also appreciated WUE only increases up to peak levels dependent on crop type and variety (Tang et al., 2014), provided heat and soil moisture stress are kept low, and management practices enhance attainment of maximum potential. It was also interesting to note field crops produced in the desert climate characterised by generally dry soils and high air temperatures still had significantly higher WUE than crops produced in the temperate regimes.

### *2.5.2.2 Amount of water supplied*

The result of Figure 2.7 suggests that, regardless of crop type and environmental conditions such as air temperature and soil type, crop WUE tends to be significantly higher in both low and high watering regimes than moderate watering regime. This result was surprising because low watering was expected to result in lower WUE than moderate watering because moisture stress was expected to be higher. However, deliberate moisture stress in crop production can be beneficial dependent on its timing during the growth cycle. Thus, moisture stress in the early growth stages of cereals is known to enhance deep rooting (Çakir, 2004; Chemura et al., 2014), essential for anchorage and reaching out to deeper horizons for soil moisture and nutrients. The high variation of WUE in the low water category suggests that some crop types indeed benefit from moisture stress. However, the most ideal practice remains the supply of adequate water complemented by good management practices for optimal growth and yield levels. Our results are consistent with findings by other studies. Chibarabada et al. (2015), when evaluating effect of watering regime on groundnut WUE, reported higher WUE under stressed than well-watered conditions. However, Boutraa et al. (2010), examining the effect of moisture stress on wheat WUE in Saudi Arabia found higher WUE in well-watered than moisture stressed wheat. Therefore, the impact of watering regime on WUE, indeed, also depends on other factors including crop type (Wajid et al. 2007; Chibarabada et al., 2015).

### *2.5.2.3 Soil texture*

Soil texture is one of the most important property of soil which greatly affect WUE and crop production (Mojid et al., 2012). Soil clay content appeared to have a significant impact on crop WUE ( $r=0.20$ ) (Table 2.5), but ideally high clay soils tend to depress crop yields. In clayey soil, maize had highest WUE than all crops (Figure 2.9A), mainly because maize does well in

most soil types (Bennetzen and Hake, 2008). High clay content soils promote waterlogging conditions, which have negative effects on most crop yields due to poor root metabolic activities (Greenway et al., 2006; Morales-Olmedo et al., 2015). Moreover, high soil water levels in high clay content soils do not always result in high water availability (Reichert et al., 2009). Poorly drained soils may also promote proliferation of fungal diseases. In addition, high clay content presents other challenges to crop production such as crusting and restrictions on root development, which are detrimental to crop yield. Most crops exhibited high WUE under loam, silt clay loam and clay loam soil compared to clay soil (Figure 2.9A-9D). WUE of wheat was higher in both silt loam and loam soil compared to clayey soil (Figure 2.9A-9C), as loam textured soils are said to be highly suitable for growing wheat and other crops (Russell, 2002). Naider and Mastorilli (2009) also found out that WUE of potato, maize, sunflower and sugar beet was significantly higher in a loam soil compared to a clay soil.

## 2.6 Conclusions

The study elucidated the impacts of crop types and environmental conditions on water use efficiency using data from 546 sites across the globe. It was concluded that field crop type had significant impact on water use efficiency with C4 plants exhibiting significantly higher efficiency than C3 types. Climate and growing season, through their effects on heat and soil moisture stress, also showed significant impact on water use efficiency with warm and wet climates or seasons resulting in higher efficiency than cooler environments. Soil texture and organic carbon content also showed positive influence on water use efficiency, most likely, through their impact on soil water availability. As soils with moderate pore sizes such as loam textured soil and soils with higher organic matter have higher water retention potential. On the other hand, water use efficiency tended to be lowest in densely populated cropping systems of cooler environments. However, high variability of water use efficiency with and across crop types as well as environmental conditions suggests more still needs to be understood about the main controlling variables of field crop water use efficiency. The studies reviewed were only looking at the WUE for grain yield and biomass production, leaving out the water use efficiency for plant carbon stocks production which is a crucial component for improving SOM. Therefore, the study recommends the inclusion of carbon stocks production component in WUE experiments. The knowledge generated from this meta-analysis is also expected to provide insights which are useful for policy and decision making in the context of climate uncertainties.



## **CHAPTER THREE**

# **EFFECT OF CROP RESIDUE QUALITY ON ITS DECOMPOSITION, MINERALIZATION AND CARBON SEQUESTRATION POTENTIAL: A LITERATURE REVIEW**

### **3.1 Introduction**

Soil nutrient depletion and land degradation have been considered serious threats to agricultural productivity and are some of the major causes of decreased crop yields in Sub-Saharan Africa (SSA) (Henaio and Baanante, 2006). Soils are an integral component of agriculture and serve as medium for numerous biological, chemical and physical processes. Soils in Africa are typically highly variable in fertility and in how they respond to inputs (Hossner and Juo, 1999). Poor cultivation practices (i.e. over tilling, mono-cropping) have resulted in decline in soil fertility and reduction of soil organic matter (SOM) (Aihou et al., 1998). To correct this, inorganic fertilizers have been used since the 1940's. This has achieved a considerable level of success over the years by increasing crop production at accelerated and balanced rates. However, application of inorganic fertilizers has also faced important limitations due to high costs, highly variable nature of soils and declined soil fertility (AGRA, 2012). The excessive use and poor management of mineral fertilizers has also resulted in soil acidification and water pollution. Therefore, organic resources have been identified as reliable alternatives to reduce continued use of inorganic fertilizers.

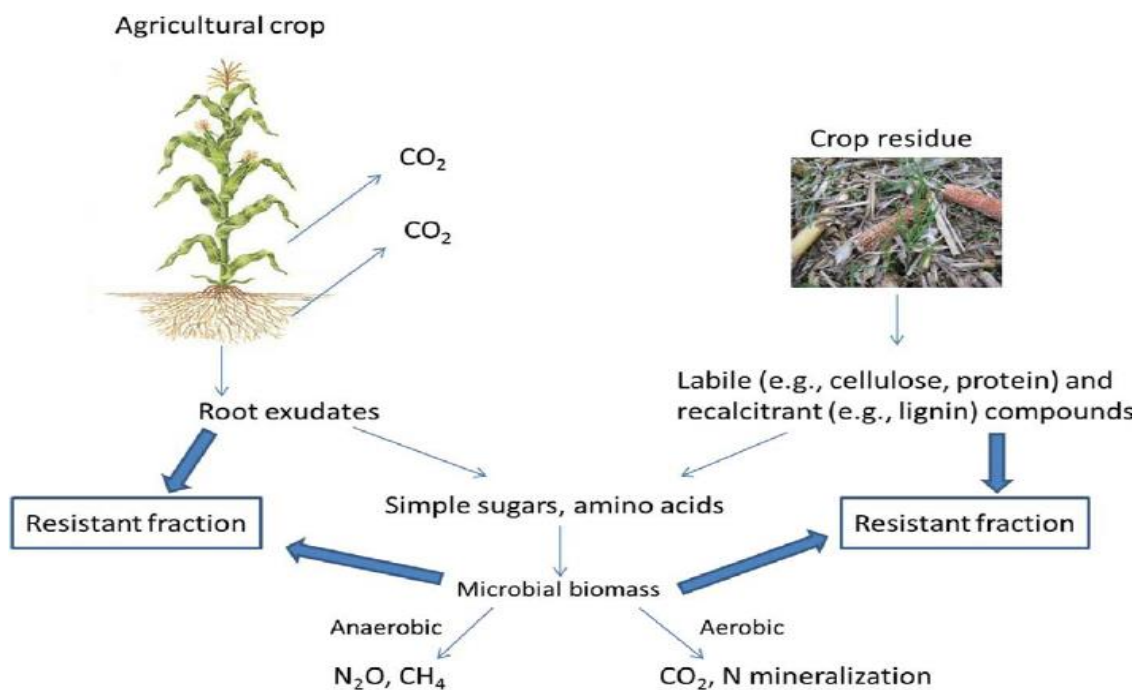
Common organic sources include crop residues, leguminous cover crops, green manures, animal manures, mulches and household wastes (Hossner and Juo, 1999). Crop residue wastes are convenient sources of organic nutrients as they are readily available to most farmers, hence

they help to improve the overall nutrient status of soils. Their retention in agricultural soils is a means of sustaining both soil organic matter levels and the pool of soil organisms, thus enhancing biological diversity and activity (Turmel et al., 2014). Crop residues contribute directly to the building of soil organic matter (SOM), which itself performs diverse functionary roles in improving the physical, chemical and biological composition of the soil (Woomer and Swift, 1994). It is estimated that about 74 Tg of crop residues are produced annually worldwide (Kim and Dale, 2004), and they are an important source of macronutrients (C, N, P and S among others) (Kumar and Goh, 2000). Nutrient release from crop residues and its cycling have influence on crop yield and can reduce the need for external inputs such as mineral fertilizers (Turmel et al., 2014). However, crop residue decomposition and nutrient release patterns are affected by physical and chemical characteristics of the residue (Palm and Rowland 1997; Singh et al., 2004), as well as environmental conditions of that area. Knowledge of the decomposition and mineralization patterns of residues are crucial to compute their contribution to soil fertility enhancement. Therefore, this paper reviews the effect of residue quality on its decomposition, mineralization and carbon sequestration potential.

### **3.2 Crop residue decomposition in the soil**

According to Juma (1999), decomposition is a biological process that includes the physical breakdown and biochemical transformation of complex organic molecules of dead material into simpler organic and inorganic molecules. It is one of most crucial processes resulting in carbon and nutrient cycling in soil. During the decomposition process CO<sub>2</sub> is released while both carbon and nutrient compounds are leached from the residues (Cotrufo et al., 2010) resulting in soil organic matter build-up. Cop residues contain complex carbon compounds in their cell wall classified into hemicellulose, cellulose, phenolic compounds and lignin based on

their molecular size, solubility and primary constituent (Figure 3.1). Residue decomposition involves physical, chemical, and biological processes and when crop residues fall onto the soil surface, soluble organic substances such as sugars and polyphenols are leached by water (Couteaux et al., 1995). This process is mostly dependent on climatic conditions (i.e. temperature, rainfall amount), microbial activity, as well as soil management practices such as cultivation.



**Figure 3. 1:** Conceptual decomposition process of crop residues in soil (Whalen et al., 2014)

Physical breakdown of crop residues into smaller parts through soil perturbations caused by human and faunal activity provide greater surface area for further microbial colonization and degradation. Easily degradable sugars, low-molecular-weight phenolic compounds and some nutrients are readily lost from the litter as they serve as easy sources of C and energy to the degrading microbes. After this, the more complex organic substrates such as cellulose, hemicellulose, and lignin undergo chemical alteration by microbes that include fungi,

actinomycetes and bacteria (Fioretto, et al., 2005). As a result, plant residue composition changes during decomposition. The final products of residue decomposition include carbon dioxide, microbial biomass, inorganic nutrients and re-synthesized organic carbon compounds such as humus, phenolics, celluloses, hemicelluloses and lignin (Cotrufo et al., 2010). Under aerobic conditions, microbial decomposition results in release of CO<sub>2</sub> and N mineralization (Figure 3.1). Under anaerobic or oxygen-limited conditions, anaerobic decomposers produce organic acids, methane and nitrous oxide (Figure 3.1).

### **3.3 Factors affecting crop residue decomposition and their impact on mineralization**

#### ***3.3.1 Impact of biochemical composition of crop residues on their decomposition***

Biochemical properties of plant residue have great influence on their decomposition. Chemical components such as carbon (C), nitrogen (N), polyphenols, cellulose and lignin content are good indicators of plant residue quality and its ease of decomposition (Loranger et al., 2002; Palm and Rowland 1997). Soil microorganisms which decompose organic matter obtain C, energy, N, phosphorus (P) and sulphur (S) from the process for building their cellular structures (Cotrufo et al., 2010). These organisms have been reported to use about 30 parts C for each part of N, so an initial C/N ratio of  $\leq 30$  promotes rapid decomposition. According to Baldock (2007), plant residues with a high C/N ratio ( $>40$ ) are mineralized far more slowly than residues with a ratio less than 40. Polyphenols are phytochemicals that are relatively resistant to decomposition. The polyphenol content may vary within the same plant material (Haynes, 1986), with roots and stem having high polyphenol content than leaves (Khan et al., 2016).

Aboveground crop residues (e.g. leaves and stems) are considered of high quality compared to belowground residues, which are relatively recalcitrant to decomposition e.g. roots (Bertrand

et al., 2006). The recalcitrant root residues are decomposed slowly due to their high lignin concentration levels and therefore contribute largely to SOM build up (Johnson et al., 2014). Sivapalan et al. (1985) found that plant residue decomposition rate decreases as the concentration of polyphenols, celluloses, and waxes increases because of enzyme inhibition and binding of mineralized N to insoluble organic compounds. Palm and Sanchez (1991) found that N mineralization was negatively correlated with polyphenol concentration ( $r=-0.63$ ) and Kaleem Abbasi et al. (2015) found that N mineralization was negatively correlated with polyphenol/N ratio ( $r=-0.73$ ,  $p\leq 0.05$ ) as well as (lignin+polyphenol)/N ratio ( $r=-0.70$ ,  $p\leq 0.05$ ). This means that plant residues high in polyphenols have low N mineralization due to the formation of stable polymers between polyphenol and amino groups. The lignin content of plant material has also been observed to be an important controlling factor on the rate of litter decomposition (Cotrufo et al., 2010). Berg et al. (1987) in an analysis of litter mass-loss rate compared with lignin concentration, concluded that high lignin concentrations were related to lower decomposition rate. Kaleem Abbasi et al. (2015) found that N mineralization was negatively correlated with lignin content ( $r=-0.84$ ,  $p\leq 0.01$ ).

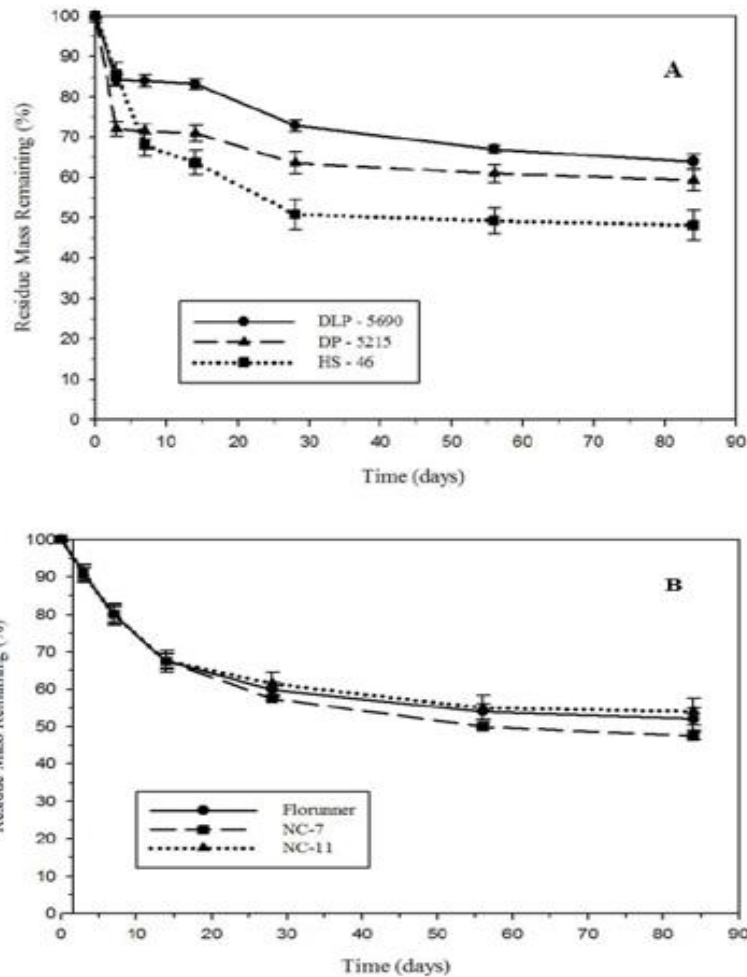
In a study by Diack and Stott (2016) which was looking at the effect of crop type and cultivar surface area on rates of decomposition in soils (chemical composition presented in Table 3.1). Lignin, hemicellulose and sugar concentration were found to control the decomposition rate of different cultivars. Significant difference was observed in lignin, hemicellulose and total N concentration of cotton and peanut. Cotton cultivar DLP-5690 exhibited higher total N, lignin and lower sugar concentration than the other two cotton cultivars (Table 3.1). Peanut cultivar Florunner exhibited higher lignin, hemicellulose and lower total N concentration but there was no significant difference in sugar concentration and total carbon content between the peanut cultivars (Table 3.1).

**Table 3. 1:** Chemical composition of aboveground biomass for different cotton and peanut cultivars (Diack and Stott, 2016)

<b>Crop</b>	<b>Cultivar</b>	<b>Total C</b>	<b>Total N</b>	<b>Sugars</b>	<b>Hemicellulose</b>	<b>Lignin</b>
-----g kg <sup>-1</sup> -----						
<b>Cotton</b>	DLP-5690	448.9a	31.4a	18.1c	252.4b	112.1a
	DP-5215	437.1b	19.3b	23.1b	133.1c	80.7c
	HS-46	457.3a	30.9a	34.0a	262.5a	103.3b
<b>Peanut</b>	Florunner	450.4a	13.4b	89.9a	176.6a	64.8a
	NC-7	455.2a	20.0a	87.7a	140.0b	42.3c
	NC-11	450.4a	18.8a	66.8a	108.2c	50.4b

Cotton cultivars exhibited significantly different rate of mass loss (Figure 3.2A). Cultivar H-46 showed higher cumulative mass loss followed by DP-5215 which was followed by cultivar DLP-5690 (Figure 3.2A). The lower residue mass loss of cultivar DLP-5690 was due to that it exhibited the highest lignin concentration than cultivars DP-5215 and HS-46 (Table 3.1) and lignin is known to be resistant to degradation thus slow down the rate of residue decomposition. In all peanut cultivars (Figure 3.2 B), the cumulative mass loss was quite high due to the lower lignin concentration combined with lower sugar concentration which was available to microorganism for degradation. They also reported that the rate of breakdown of the aboveground residues between the peanut cultivars was not significant different because of the insignificance difference in their sugar concentration (Table 3.1). Therefore, from these findings it can be confirmed that the decomposition patterns of different cultivars of the same crop also depend on the biochemical quality. Their findings also showed that high lignin residues such as the cotton cultivar DPL-5690 (Figure 3.2 A) will have slower conversation to soil organic matter than those with lower lignin and will be good SOM sources in long term. More specifically, they indicated that the effect of lignin concentration on litter mass-loss rates may be described as a negative linear relationship in the later stages of decomposition (Berg and McLaugherty, 2007). Lignin: N ratio is also taken as an efficient indicator of litter

decomposition rate. In other words, lignin's control on decomposition rate will be stronger in high lignin residues than in low lignin residues, thereby slowing down decomposition or the release of mineral nutrients.



**Figure 3. 2:** Changes in aboveground residue mass loss over time for (A) cotton and (B) peanut cultivars. Bars represent standard deviation over time (Diack and Stott, 2016)

### 3.3.2 Impact of physical nature of the residues on their decomposition

Compared to chemical quality there is much less attention paid to physical quality of crop residues on their decomposition rate. Plant physical properties include particle size and surface properties, these have potential to affect the accessibility of residues to soil organisms, and thus alter rates of colonization and decomposition patterns. It has been reported that smaller

particles decompose faster than larger particles because smaller particles have a greater surface area to volume ratio, allowing greater access to soil microorganisms (Summral and Burgess 1989). In a study by Singh et al. (2004), CO<sub>2</sub>-C emission from soil amended with <1 mm canola (*Brassica napus* L) residues was significantly smaller (p<0.05) than that amended with 20-25 mm and 5-7 mm residues of canola. The slower decomposition of grounded residues (<1 mm) were surprising because by reducing the size of canola residues, an increase in C mineralization was expected due to their greater distribution in the soil and hence greater accessibility to microbial attack. However microbial sulphur in their study was not affected by the size of canola particles as it was about the same under all canola sizes. However, in a study by Pal and Marschner (2016), residues of kikuyu grass and wheat with smaller particles size (0.2-2 mm) were decomposed faster than residues with larger particles (3-4 mm).

In another study by Angers and Recous (1997), the decomposition of wheat straw and rye residues as affected by particle size was studied. It was reported that after incubation, the very fine particles of wheat and rye (<0.1 cm) showed greatest amount of C mineralized whilst the intermediate size classes (0.5 and 1 cm) showed the lowest amount of C mineralized. The greater availability and accessibility of finer particles to microbes was reported to be responsible for the higher rates of decomposition observed for finely ground residues. In contrast, in a study by Bending and Tuner (1999), decomposition depended on the chemical quality of crop residue material with residues of lower C: N ratio decomposing faster than those of high C: N ratio regardless of particle size.



### **3.4 Impact of soil properties on residue decomposition**

#### ***3.4.1 Impact of chemical soil properties on residue decomposition***

Soil chemical properties include pH, organic matter content, and nutrient availability; all of which influence the composition of the soil microbial population. Soil pH has been reported to affect density and the activity of microorganisms involved in the process of decomposition and thereby the rate of decomposition of organic matter (Haslam and Tibbett, 2009). The rate of decomposition is greater in neutral soils than in acidic soils, as microbial activity is greatest at neutral soil pH. According to Allison (1973), high pH ( $\text{pH} > 8$ ) and high concentration of nitrogen in the soil will favour multiplication of bacteria, while low pH ( $\text{pH} < 6$ ) and low soil nitrogen concentration will favour the growth of fungi. Therefore, treatment of acid soils with lime can accelerate bacterial decomposition.

Baath et al. (1979) found that acidification decreased the decomposition rate of pine needle and root litter, and there were significant changes on microbial properties due to acid treatment. Thus, FDA-active fungal lengths as well as bacterial numbers and cell sizes decreased in the acidified plots compared to control. Soil nutrient availability has also been identified as one of the controlling factors affecting the rate of litter decomposition (Swift et al., 1979). Liu et al., (2006) found that additions of N in the form of urea fertilizer and P in the form of triple superphosphate increased decomposition rate of residues of the perennial herb *Allium bidentatum* (N= 5.0 g/kg; C: N=97) and the grass species *Stipa krylovii* (N=2.9 g/kg; C: N=174). In addition, *Allium bidentatum* residues had higher decomposition rate than *Stipa krylovii* residues which could be possibly due to *Allium bidentatum* having lower C: N ratio than *Stipa krylovii*. Kwabiah et al. (1999) suggested that responses of plant litter decomposition to soil nutrients were determined by litter quality.

### ***3.4.2 Impact of physical soil properties on residue decomposition***

Soil texture is the most important and influential soil physical property determining residue decomposition amongst the other soil physical properties, such as bulk density, porosity, soil structure and consistency. It controls nutrient and water dynamics, surface area, and other soil properties. To understand the relationship between soil texture and organic matter dynamics, some studies focused on determining the possible mechanisms of textural controls on SOM dynamics. Mtambanengwe et al. (2004) looked at the decomposition of organic matter in soils as influenced by texture and pore size distribution. In this study, carbon mineralization of tobacco starch (*Nicotiana tabacum*) and barley straw (*Hordeum vulgare*) decreased with increasing clay content, with ranges of 42-121 mg C g<sup>-1</sup> soil in a 5.6% clay soil and 34-107 mg C g<sup>-1</sup> soil in 56% clay soil. Micropores of diameter <75µm in the 56% clayey soil were reported to be responsible for the protection of organic substrates against microbial attack resulting in lower carbon mineralization. This study provided empirical evidence to support the theory that decomposition of fresh organic matter is governed by its physical accessibility by microbes as determined by soil texture and pore size distribution.

### **3.5 Impact of climatic conditions on residue decomposition**

Residue decomposition is a biological process, it is therefore sensitive to environmental conditions such as temperature and moisture. Water availability, more especially in the early stages of decomposition have been found to influence the rates of crop residue decomposition and nutrient release since it affects the activities of decomposer organisms (Liu, et al., 2006). Water availability in the form of rainfall can also affect decomposition through leaching and break down of surface residue (Swift, et al., 1979). In general, residue moisture content of more than 150 % or below 30 % (dry weight basis) tend to slow residue decomposition (Haynes,

1986). Within this range, decomposition rates will increase with increasing moisture if temperature is adequate (Bunnell, et al., 1977). Furthermore, decomposing organisms have a wide range of optimal temperatures of 0 to 45°C (Paul, 2006), even though their activities often show a positive correlation with increased temperature (Swift, et al., 1979).

In a study by Al-Kaisi et al., (2017), which looked at the effect of nitrogen fertilizer application and temperature on corn residue decomposition. The treatments included corn residue samples treated with different N rates (0 kg N ha<sup>-1</sup>, 34 kg N ha<sup>-1</sup> or 67 kg N ha<sup>-1</sup>) of 32% liquid solution of urea and ammonium nitrate (UAN) at each incubation temperature (0°C, 25°C and 35°C). The N applications in the incubation study did not show significant effect on residue decomposition and on cumulative CO<sub>2</sub>-C evolution. The driving force for increasing residue decomposition was temperature, with greatest rate of CO<sub>2</sub>-C evolution and cumulative CO<sub>2</sub>-C release occurring at 25°C. The amount of CO<sub>2</sub>-C released at 35°C was lower compared to that released at 25°C, because greater amount of mineralizable (labile) C had decomposed at 25°C. However, numerous manipulative experiments demonstrate that increased temperature results in higher rates of CO<sub>2</sub>-C evolution and residue mass loss (Gudas et al., 2010; Wang et al., 2004).

### **3.6 Residue decomposition effect on soil C sequestration and nutrient mineralization**

#### ***3.6.1 Soil carbon sequestration potential of crop residues***

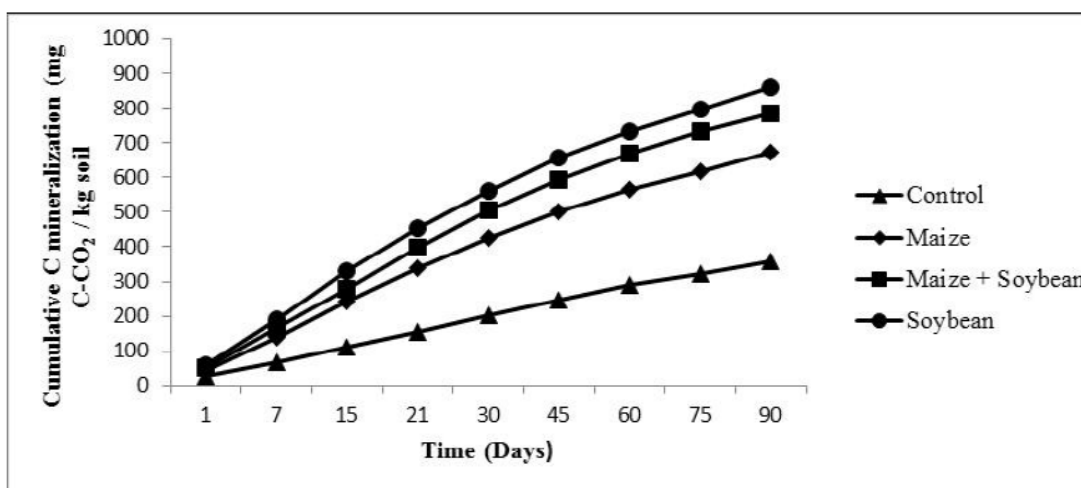
Residue decomposition is considered as the release of carbon dioxide and leaching of compounds, including both carbon compounds and nutrients (Cotrufo et al., 2010). Therefore, for carbon to be added into the soil during residue decomposition, there must be less CO<sub>2</sub> emission. Carbon dioxide is an important greenhouse gas accounting for 60% of the total

greenhouse effect (Giacomini et al, 2007). It is well known that vegetation and soils are major storage sinks of atmospheric CO<sub>2</sub> (Gholz et al., 2000). The organic carbon pool in agricultural soils can enhance agricultural sustainability and serve as a potential sink of atmospheric CO<sub>2</sub> (Zhang et al., 2008). Soil C sequestration improves soil quality and reduces the contribution of agriculture to CO<sub>2</sub> emissions. However, the decomposition and CO<sub>2</sub> release by organic residues have been found to depend on biochemical quality of the residues or their particle size. In a study by Gezahegn et al. (2016) where the decomposition and nitrogen mineralization of individual and mixed maize and soybean residue were studied. Maize residue exhibited higher concentration of organic C, hemicellulose, cellulose, lignin and lower C: N ratio compared to soybean residue and maize+soybean residue (Table 3.2).

**Table 3.2:** Chemical properties of maize, soybean residue and their mixture (Gezahegn et al., 2016)

Values in unit±SD	Organic C	Total N	Hemicellulose	Cellulose	Lignin	C: N
	-----%-----					
<b>Maize residue</b>	41.5±0.43	1.18±0.03	20.6±1.90	31.9±1.97	4.37±0.55	35.3±1.32
<b>Soybean residue</b>	37.2±0.63	3.07±0.24	17.1±2.72	26.8±2.13	2.89±0.74	12.3±0.74
<b>Maize+soybean residue</b>	40.3±0.64	2.02±0.45	18.8±1.49	29.3±2.22	3.47±0.62	18.8±1.49

Soybean residue had significantly higher cumulative C mineralization throughout the incubation period, while maize residues had lower C mineralization than soybean and the mixture (Figure 3.3). The higher C mineralization in soybean residues compared to maize was attributed to higher N content and lower C: N ratio of soybean residues (Table 3.2). In contrast the slower decomposition and lower C mineralization in maize residue amended soil was reported to be due to higher lignin and hemicellulose contents of maize.



**Figure 3.3:** Carbon mineralization of maize, soybean and their mixture in a 90 day incubation study (Gezahegn et al., 2016)

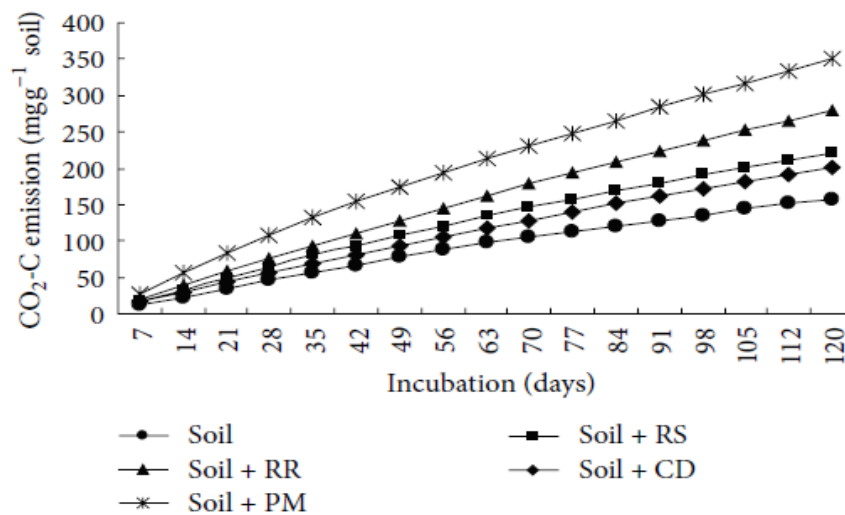
Hossain and Pute (2013) also compared emissions of CO<sub>2</sub> by different organic material namely rice straw, rice root, cow-dung and poultry litter (biochemical properties presented in Table 3.3).

**Table 3.3:** Characteristics of organic residues used in a decomposition study, (Hossain and Pute, 2013)

Parameters	%C	%N	%P	%K	C:N ratio
Soil	0.92	0.08	0.0098	0.0025	11.5
Rice straw	48.90	0.63	0.08	2.35	77.61
Rice root	42.20	0.40	0.29	0.34	105.5
Cow dung	17.43	1.04	0.82	0.68	16.75
Poultry manure	47.41	1.00	0.69	0.95	47.41

They reported that cow-dung treated soil produced the lowest CO<sub>2</sub>-C compared to other organic sources (Figure 3.4), which might have been due to its low initial C content (Table 3.3). It was explained that cow-dung is a well decomposed organic material, as a result it has less amount of labile C for producing CO<sub>2</sub>-C after incorporation in soil. Higher CO<sub>2</sub>-C emission was observed in poultry manure (Figure 3.4). This might be due to its high initial C content as well

as the fact that it had finer material than other organic residues which favoured bacterial activity for decomposition.



**Figure 3. 4:** Cumulative CO<sub>2</sub>-C evolution from different organic sources. Soil + RR= soil with rice root residue, Soil + PM= soil with poultry manure, Soil +RS= soil with rice shoot residue, Soil +CD= soil with cow dung (Hossain and Pute, 2013)

In a study by Abro et al. (2011), nitrogen fertilizer was applied at rates of 40 (N<sub>1</sub>), 80 (N<sub>2</sub>), 160 (N<sub>3</sub>) and 320 (N<sub>4</sub>) mg N kg<sup>-1</sup> to adjust the C: N ratios of maize (*Zea maize* L.) straw to 80, 40, 18 and 9 respectively. This was done to compare the decomposition characteristics of maize residues with different C: N ratios under four different moisture regimes (60%, 70%, 80% and 90% field capacity). C: N ratio of 18 (N<sub>3</sub>) and moisture level of 70% and 90% field capacity resulted in higher CO<sub>2</sub>-C production while C: N ratio of 9 (N<sub>4</sub>) resulted in lower CO<sub>2</sub>-C production at all moisture regimes. The reduced CO<sub>2</sub>-C at N<sub>4</sub> dose showed that excess N could reduce CO<sub>2</sub> production and that N can only enhance CO<sub>2</sub>-C evolution up to a certain rate, otherwise reduction will occur. At N<sub>4</sub> the reduced CO<sub>2</sub>-C evolved could be due to luxurious consumption of N by soil microbes that suppressed CO<sub>2</sub>-C production or N mineralization per

unit CO<sub>2</sub>-C evolved when N was in abundance. They also found that retaining maize straw in the soil significantly increased soil organic carbon (SOC) and microbial nitrogen contents (MBN), which could be attributed to enhanced microbial activity by mixing of straw with soil. Soil microbes decompose organic residues through degradation and transformation, enhance SOM and nutrient cycling and are a living index that reflect soil fertility and environmental quality.

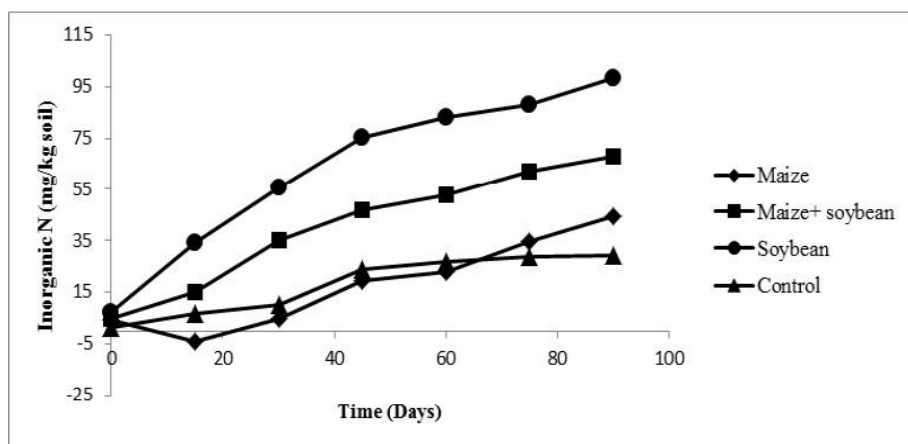
### ***3.6.2 Impact of residue decomposition on nitrogen and phosphorus mineralization in soil***

Incorporating plant residues into agricultural soils does not only sustain organic C content, but also increases nutrient availability (Turmel et al., 2014). Incorporation of crop residues provides readily available N, S and P to soil depending upon the decomposition rate of material (Murungu et al., 2011). N availability from the residues depends on the amount of N mineralized or immobilized during decomposition. Inorganic N in soils is predominantly NO<sub>3</sub><sup>-1</sup> and NH<sub>4</sub><sup>+</sup>. Recent research has indicated that the properties of the retained plant residues influence the inorganic soil nitrogen concentrations.

High quality residues often result in high N mineralization rates. These are characterized by high N content in their tissues and lower C: N ratios and can be decomposed faster in comparison with low quality residues (Sanchez 2001). A study by Kaleem Abbasi et al., (2015) assessed the impact of addition of different plant residues on nitrogen mineralization-immobilization turnover and carbon content of a soil incubated under laboratory conditions. Their findings deduced that shoots of *Glycine max*, as well as shoots and roots of *Trifolium ripens* resulted in continuous N mineralization by releasing a maximum of 109.8, 74.8 and 72.5 mg N kg<sup>-1</sup> respectively; representing a 55, 37 and 36% recovery of N from the soil. These residues exhibited substantial mineralization potential, demonstrating that legumes and trees

can produce high-quality residues that have the potential to promote N cycling in agro-ecosystems.

Also, in a study by Gezahegn et al. (2016) where the decomposition and nitrogen mineralization of maize and soybean residues were studied (biochemical residue quality represented in Table 3.2). All treatments generally showed increases in mineral N with time. The addition of soybean and maize + soybean residue resulted in significant increase in inorganic N compared to the control (Figure 3.5). In contrast, addition of maize residue resulted in a significant lower inorganic N compared to the control until day 60 of incubation. However, at the end of the incubation the N released from maize residue decomposition was higher than that released from the control. The higher N mineralisation by soybean residues was attributable to its low C: N ratio, low lignin and hemicellulose content in its residues which enables it to decompose rapidly releasing large amounts of N during decomposition (Table 3.2). On the other hand, lower inorganic N pool in maize straw treated soil than control in the first 60 days of incubation was reported to be due to the fact that growing microbial populations depleted the available pools of N to compensate for the decomposition of low N maize residues.



**Figure 3.5:** Patterns of N released from maize, soybean and their mixture during a 90 day incubation study (Gezahegn et al., 2016).



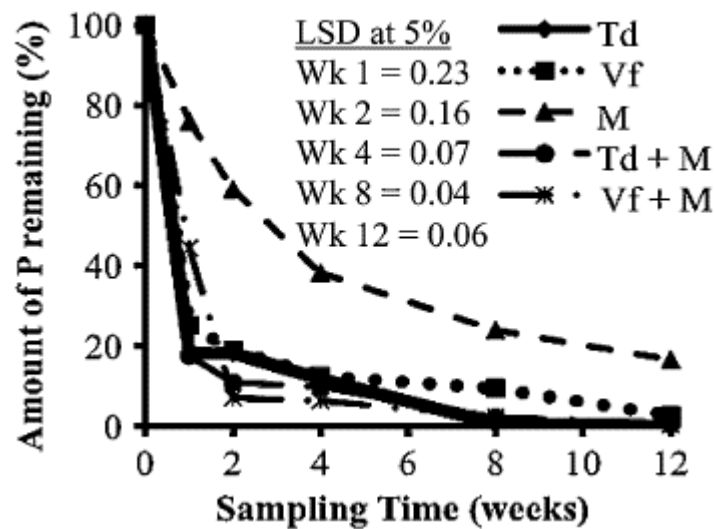
Phosphorus is another most important soil element required for the production of healthy crops (Sharma et al., 2013). It is found in different forms including inorganic, organic and microbial phosphorus. Organisms need phosphorus for metabolic activity and as a structural component of many biochemical compounds. Only P in the soil solution is available for plant uptake, but its concentration is usually low because of precipitation and immobilization which strongly affect plant available P in soil (Sharma et al., 2013). During residue decomposition, organic P in plant residues is mineralized by phosphate enzymes which are produced by microorganisms, plants and mycorrhiza (Terafdar and Jungk 1987). The amount of P in the soil solution mainly depends on P released by the incorporated residues. Kwabia et al. (2003) investigated the relationship between plant residue quality on available P as well as microbial biomass P and C. They concluded that the initial increase of available P resulted from release of soluble P from plant residues. A litter bag study by Partey et al. (2013) investigated maize residue interaction with high quality organic materials, their effects on decomposition and nutrient release dynamics. The decomposition study was performed using the aboveground portions of *Vicia faba* (Vf), *Tithonia diversifolia* (Td) and *Zea mays* (M) either in sole or mixed treatments, with biochemical properties presented in Table 3.4.

**Table 3.4:** Chemical composition of sole and mixed organic residues (Partey et al., 2013)

Treatment	N	P	K	C	Ca	Mg	Lig	Poly	C:P
.....g/kg.....									
<b>Td</b>	28.1 (0.8) <sup>bc</sup>	5.2 (0.2) <sup>c</sup>	46.2 (1.4) <sup>c</sup>	400.6 (4.2) <sup>a</sup>	13.0 (1.1) <sup>c</sup>	8.3 (0.2) <sup>c</sup>	58.0 (1.8) <sup>c</sup>	18.0 (0.7) <sup>d</sup>	77.0
<b>Vf</b>	54.7 (1.0) <sup>d</sup>	2.5 (0.2) <sup>a</sup>	17.6 (0.3) <sup>a</sup>	427.4 (2.4) <sup>cd</sup>	27.0 (1.1) <sup>e</sup>	3.0 (0.3) <sup>a</sup>	41.0 (1.4) <sup>a</sup>	14.0 (0.8) <sup>c</sup>	171
<b>M</b>	10.8 (0.6) <sup>a</sup>	2.9 (0.1) <sup>a</sup>	20.6 (0.7) <sup>a</sup>	401.3 (4.4) <sup>ab</sup>	4.2 (0.1) <sup>a</sup>	2.9 (0.1) <sup>a</sup>	57.0 (1.9) <sup>c</sup>	5.6 (0.3) <sup>a</sup>	138.4
<b>Td+M</b>	25.4 (1.2) <sup>b</sup>	4.3(0.1) <sup>b</sup>	33.4 (0.7) <sup>b</sup>	417.6 (5.1) <sup>bc</sup>	8.2 (0.6) <sup>b</sup>	6.3 (0.1) <sup>b</sup>	56.7 (1.3) <sup>c</sup>	10.2 (1.0) <sup>b</sup>	97.1
<b>Vf+M</b>	31.3 (0.8) <sup>c</sup>	2.7 (0.2) <sup>a</sup>	19.4 (0.3) <sup>a</sup>	436.2 (1.5) <sup>d</sup>	19.7 (1.0) <sup>d</sup>	2.8 (0.2) <sup>a</sup>	48.0 (1.5) <sup>b</sup>	8.1 (0.4) <sup>ab</sup>	161.6

Values are the means of four replicates. Values in parentheses are standard errors of means. Values with the same letters as superscript do not differ significantly according to Tukey test at 5 % probability level  
Lig-lignin, Poly-polyphenol, Td-*T. diversifolia*, Vf -*V. faba*, M- *Zea mays*

Compared with *V. faba* and *T. diversifolia* biomass, *Z. mays* was found to be of low quality based on its chemical composition as presented in Table 3.4. It exhibited low concentration of N, and high lignin and C: P ratio. As a result, P release rate was significantly lowest in sole *Z. mays* ( $p < 0.05$ ) throughout the decomposition period (Figure 3.6). Lupwayi et al. (2003) reported that P release from crop residues is influenced not only by initial P content of the residue, but also its ease of decomposition. Therefore, *Z. mays* released the least P simply because it had lower initial P content, high lignin content and C: P ratio in its tissues which induced immobilization of P thus slow decomposition.



**Figure 3.6:** P release patterns in decomposing sole and mixed plant materials of *T. diversifolia* (Td), *V. faba* (Vf) and *Z. mays* (M) over 12 weeks placement in soil. Data points are means of five replicates (Partey et al. 2013).

### **3.7 Conclusion**

Plant residue decomposition is a continuous and complex process, which is controlled by the biochemical quality of residues, soil properties and environmental factors. From the literature reviewed, it can be concluded that decomposition of organic material in the soil is mainly controlled by the biochemical properties of the organic material. Organic material with lower C: N ratio, higher nitrogen content and lower lignin content will decompose fast emitting CO<sub>2</sub> into the atmosphere and releasing higher mineral nitrogen into the soil. On the other hand, organic material with higher C: N ratio will decompose slowly thereby sequestering more carbon into the soil. Again, organic material with finer particles will decompose faster and release higher CO<sub>2</sub> compared to materials with coarse particles. In addition, higher amounts of clay in soil will promote greater protection of organic material in micropores, thereby shielding them from microbial attack. This results in greater carbon sequestration in fine-textured soil compared with coarse textures. In all, residue decomposition is a beneficial process since it improves soil fertility by availing mineral nutrients such as N and P, while soil C is sequestered.

## CHAPTER FOUR

### SELECTION OF WHEAT (*Triticum aestivum*) GENOTYPES FOR IMPROVED WATER USE EFFICIENCY FOR GRAIN YIELD, BIOMASS AND ATMOSPHERIC CARBON SEQUESTRATION

#### 4.1 Abstract

Plants have great potential to sequester soil organic carbon thereby mitigate climate change and improve soil fertility. Some studies have focused on the ability of crops to store C and allocate it to roots, and on WUE for production of grain and biomass, while the variations between crop genotypes and water use efficiency of plant carbon stocks have received less attention. The objective of this study was to compare WUE for grain yield, plant biomass as well as plant carbon stocks of selected wheat genotypes under different soil water regimes. The experiment was set up under field and greenhouse conditions using 100 wheat genotypes from CIMMYT. These were grown at 25% (water-stressed) and 75% (non-stressed) field capacity (FC) using an alpha lattice with 10 blocks and 10 genotypes per block. Treatments were replicated twice in the field and three times in the glasshouse. Results showed that average wheat grain yield (GY) varied from 326 g m<sup>-2</sup> to 2062 g m<sup>-2</sup>, average shoot biomass (SB) ranged from 1873 g m<sup>-2</sup> to 3726 g m<sup>-2</sup> while average total plant biomass (PB) ranged from 2992 g m<sup>-2</sup> to 6289 g m<sup>-2</sup>. Grain carbon stocks (GCs) averaged 132 g C m<sup>-2</sup> and 167 g C m<sup>-2</sup> in the glasshouse under stressed and non-stressed conditions respectively. The total plant carbon stocks (PCs) ranged from 691 g m<sup>-2</sup> to 3093 g m<sup>-2</sup> (i.e. 348% difference) in the glasshouse, while it was 835 g m<sup>-2</sup> to 4016 g m<sup>-2</sup> (i.e. 381% difference) in the field. Water use efficiency for grain yield production (WUE-GY) ranged from 0.12 g m<sup>-2</sup> mm<sup>-1</sup> to 2.10 g m<sup>-2</sup> mm<sup>-1</sup> (i.e. 18 fold increase) in the glasshouse under stressed conditions while it was 0.57 g m<sup>-2</sup> mm<sup>-1</sup> to 4.01

g m<sup>-2</sup> mm<sup>-1</sup> in the field under stressed conditions. WUE components varied amongst wheat genotypes. LM75 exhibited higher WUE-GY under stressed conditions while genotypes LM48 and LM47 exhibited lower WUE-GY under non-stressed conditions. LM75 and BW162 were ranked best genotypes for WUE-PCs, while BW162 was also ranked the best genotype for WUE-RCs under water-stressed conditions. Variability in storing C under different scenarios of water availability exists among the wheat genotypes studied.

**Keywords:** agronomic traits, grain yield, root to shoot ratio, water stress, carbon stocks

## 4.2 Introduction

The global climate is changing because of natural as well as anthropogenic activities (Sarangle et al., 2018). Climate change has been found to cause long-term alterations in temperature and precipitation. Natural processes such as solar radiance variations also result in fluctuations in the climate (IPCC 1996). While anthropogenic processes such as deforestation, agriculture and other land uses which contribute to carbon dioxide (CO<sub>2</sub>) emission also results in climate fluctuations since CO<sub>2</sub> is considered the primary cause of global warming (IPCC 2014). Furthermore, it has been reported that carbon dioxide concentration in the atmosphere would be doubled by 2050 if the current rate of CO<sub>2</sub> increase continues, causing the world temperature to rise by 2-4 °C (IPCC 2013). According to IPCC, without policies to attenuate GHG emissions, GHGs (which the most dominant is CO<sub>2</sub>) would increase from 580 ppm to 700 ppm by the mid of current century (Nordhous 2007).

According to Gbetibouo et al. (2010), in South Africa agriculture is the most vulnerable sector to climate change as crop production is affected by a number of factors including rainfall patterns, temperature, water availability and evapotranspiration. Carbon dioxide is regarded as the driving force for climate change. However, its direct effect on plants is positive (Warrick, 1988), as a result, they could be a potential sink for CO<sub>2</sub>. The abundance of CO<sub>2</sub> in the atmosphere can increase photosynthesis in plants (Monson 1999). Crops that exhibit positive responses to enhanced CO<sub>2</sub> are characterized as C3 plants (Leakey, 2009). Thus, increased CO<sub>2</sub> in the atmosphere decreases transpiration of C3 plants as they partially close their stomata thus minimising plant water loss (Kimball et al., 2002). Wheat is characterised as a C3 crop and is the third most produced cereal after maize and rice in the world (Wang et al., 2016). In South Africa, it is the second most important grain crop after maize, and is consumed as bread, cakes, cookies, livestock feed and alcoholic beverages (DAFF,2010). Annual wheat production in

South Africa over the past years has ranged between 1.4 and 2.1 million tons, with an average yield of 2 to 3.1 t ha<sup>-1</sup> under dryland and about 5 to 7 t ha<sup>-1</sup> under irrigation (DAFF, 2010). However South Africa's wheat production has gradually declined from 2.5 million tonnes, produced on 974 000 ha in 2002 to approximately 1.7 million tonnes, produced on 500 00 ha in 2013 (Sosibo et al., 2017). This has been associated with erratic and unevenly distributed rainfall patterns in South Africa with annual average rainfall of 495 mm, which is far below the world's average of 860 mm per year (Gbetibouo & Hassan, 2005). The decline in wheat production could also be a result of SOC depletion characteristic of most South African soils (Du Preez et al., 2011). Soil with less organic matter support poor average crop yields as they hold less nutrients and are more susceptible to drought (Schlesinger 1999).

Many studies have focused on the effect of moisture stress on wheat grain yield and measures for improving WUE of wheat (Hagyó et al., 2007, Zhang and Oweis., 1999, Mirbarhar et al., 2009). Hagyó et al., (2007) reported that moisture stress during spike emergence and anthesis stage reduced wheat grain yield by up to 20% mainly through reduction of individual grain weight. Zhang and Oweis (1999) reported that water stress at anthesis stage reduces pollination and number of grains per spike which results in the reduction of grain yield of wheat. Su et al. (2007) reported that no till and mulching increase water storage and wheat yields enhancing its WUE. While Zhang et al. (1998) found that WUE of grain yield was increased from 9.7 to 11.0 kg ha<sup>-1</sup> mm<sup>-1</sup> by supplementary irrigation. Studies focusing on WUE for plant carbon stocks production in order to select crops that are water use efficient and can sequester more C into the soil are lacking.

Agricultural soils have been identified as having significant potential to sequester soil organic carbon (SOC) thereby mitigating climate change (Lal et al., 2007). The actual amount of C sequestered depends on management strategies such as residue retention and environmental

conditions (Luo et al., 2010). Carbon is added to soil by plants that have captured CO<sub>2</sub> from the atmosphere through photosynthesis to form carbon compounds. Plant C then enters the SOC pool through decaying plant litter and/or roots, as well as through root exudates. Different crops therefore exhibit different abilities for storing carbon in their tissues. According to Mathew et al., (2017), grasses exhibited the highest total plant carbon stocks ( $6.80 \pm 0.5 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ), followed by maize ( $6.30 \pm 0.34 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ), cotton ( $4.3 \pm 0.47 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ) and soybean ( $3.00 \pm 0.48 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ ). This suggests that variation amongst crop cultivars also exists. Since wheat is a C3 crop, it has potential to adapt to increased atmospheric CO<sub>2</sub> and limited water availability. It is also an important crop to the country's economy. Therefore, there is need to select for wheat genotypes that produce high plant carbon stocks with less water use. Therefore, the present study aims at selecting wheat genotypes for improved water use efficiency for grain, biomass yield production and plant carbon sequestration.

## **4.3 Materials and methods**

### ***4.3.1 Plant materials***

One hundred genotypes, consisting of 97 drought and heat tolerant wheat accessions, as well as 2 commercial wheat varieties from France and Triticale were evaluated. The drought and heat tolerant genotypes were obtained from CIMMYT. These were used owing to their genetic variability for rooting abilities and breeding history for drought tolerance. The French varieties and Triticale were used as comparative controls because they are known for their high rooting capacities. These are winter wheat genotypes with twice the rooting capacity of wheat grown in warmer winters (Thorup-Kristensen et al., 2009), while Triticale has an aggressive root system inherited from rye (*Secale cereale*) (Gelalcha et al., 2007).



### **4.3.2 Growing conditions and trial management**

#### **4.3.2.1 Field experiment**

The field experiment was carried out from May to September 2017 at the University of KwaZulu Natal's Ukulinga Research farm (LAT: 29.667° LON: 30.406° and ALT: 811 m) using a 10\*10 alpha lattice design with two replications. Long-term average temperature and rainfall for Ukulinga are 18°C and 738 mm, respectively. The average temperature during the growing period and soil properties are given in Table 4.1. The field was ploughed to a depth of 30 cm and custom-made plastic mulch was used to exclude rainwater. Three seeds were planted per station at 10 cm intra-row spacing and 30 cm between rows soon after ploughing. Each row consisted of 10 genotypes and was treated as an incomplete block. Basal fertilizer was applied at a rate of 120:30:30 kg ha<sup>-1</sup> (N: P: K). Other agronomic practices were as per normal wheat production practice in South Africa (DAFF, 2010).

Irrigation was applied through a drip irrigation system to maintain soil water content at FC in the well-watered regime. Under the drought stress treatment, irrigation was withheld 5 weeks after crop emergence until just before signs of wilting were observed upon which irrigation was reinstated. The watering regimes for stressed and non-stressed condition were 25% FC and 75% FC, respectively. The level of soil moisture was monitored with hand held digital moisture probes model PCE-smm1. During the field experiment, irrigation was withheld before anthesis to induce drought stress in a way that simulated *in situ* wheat production under field conditions. At maturity, the aboveground biomass was cut-off the soil surface to separate the below ground biomass. Plant parts for each plot were separated into grain, shoot and root. The separated plant parts were oven dried at 60°C for 72 hours to measure the dry weight. The dry weight was converted to gram per square metre (g m<sup>-2</sup>) accordingly using the plant population of 134 plants per square metre. Root: shoot ratio (R: S) and total biomass PB (sum of grain yield, shoot biomass and root biomass) were computed. Due to greater variations amongst the 100 wheat

genotypes, the best 10 genotypes (genotypes with high total biomass) were selected for the experiment in the greenhouse.

#### ***4.3.2.2 Greenhouse experiment***

A greenhouse experiment was carried out from January to May 2018 at the Controlled Environment Facility of the University of KwaZulu Natal Pietermaritzburg Campus. This was conducted using a 10\*10 alpha lattice design with three replications. Ten seeds were sown in each pot containing soil collected at Ukulinga (properties presented in Table 4.1) and thinned to 8 plants per pot, 3 weeks after emergence. Ten pots were allocated per incomplete block and genotypes were randomly assigned to pots to minimize experimental error. The amount of water required was added manually using a watering can. Fertilizer was applied at a rate of 300 kg N ha<sup>-1</sup> and 200 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as per fertilizer recommendation of the soil used. The different water regimes were initiated 6 weeks after planting to ensure good establishment and early exposure of all growth stages to drought. In the non-stressed conditions, plants were watered to 75% field capacity (FC), while in the water stressed conditions volumetric soil water content was maintained at 25% of FC. The soil water content was monitored by a hand held soil moisture probe (PCE-smm1 model) and weighing of pots. The two watering treatments were maintained until maturity (~120 days). At maturity, which was reached after four months planting plant parts for each pot were separated into grain, shoot and root and oven dried at 60°C for 72 hrs to measure the dry weight. This was then converted to gram per square meter (g m<sup>-2</sup>) units using a plant population of 128 plants per square meter. Root: shoot (R: S) ratio and total biomass (PB) were computed after determining GY, RB, and SB.

**Table 4. 1** Soil properties and mean temperatures for the two environments used in this study

<b>Property</b>	<b>Greenhouse experiment</b>	<b>Field experiment</b>
Bulk density (g cm <sup>-3</sup> )	0.99	1.04
Phosphorous (mg/L)	29.00	39.00
Potassium (mg/L)	412.00	241.00
Calcium (mg/L)	1386.00	1453.00
Magnesium (mg/L)	504.00	369.00
Electrical Conductivity (cmol/L)	12.24	11.02
pH (KCl)	5.31	4.56
Organic carbon (%)	3.40	2.60
Nitrogen (%)	0.29	0.23
Clay (%)	33.00	28.00
Mean Temperature (°C)	20.13	16.63

pH (KCl) =pH measured on the potassium chloride

#### **4.3.3 Carbon stocks and water use efficiency (WUE) determination**

. Grain, shoot and root samples of the 10 selected wheat genotypes were then ground to <0.5 mm and analysed for total C and N in triplicates using a LECO CNS-2000 Dumas dry matter combustion. The total carbon and nitrogen contents (TC<sub>C</sub>, TN<sub>C</sub>) were then used to estimate the C stocks in the different plant parts as follows:

$$GC_S = GY \times GC_C \times b \quad (1)$$

where GC<sub>S</sub> is the grain C stock (kg C m<sup>-2</sup>), GY is grain yield and GC<sub>C</sub> is the C concentration, in the grain (g C kg<sup>-1</sup>); and b is a constant equal to 0.001.

$$SC_S = SB \times SC_C \times b \quad (2)$$

where  $SC_S$  is the shoot C stock ( $\text{kg C m}^{-2}$ ),  $SB$  is the shoot biomass and  $SC_C$  is the C concentration in the shoots ( $\text{g C kg}^{-1}$ ); and  $b$  is a constant equal to 0.001.

$$RC_S = RB \times RC_C \times b \quad (3)$$

where  $RC_S$  is the root C stock ( $\text{kg C m}^{-2}$ ),  $RB$  is the root biomass and  $RC_C$  is the C concentration in the roots ( $\text{g C kg}^{-1}$ ); and  $b$  is a constant equal to 0.001.

The **total plant carbon stocks (PCs)** corresponded to the sum of C stocks from the different plant parts ( $GC_S+SC_S+RC_S$ ).

Water use efficiency of wheat genotypes was calculated from the ratio of biomass and the total volume of irrigation water applied using the following formula:

$$WUE \text{ (g m}^{-2} \text{ mm}^{-1}) = \frac{B}{V}$$

Where **B** is the mass of either Grain (GY), Shoot Biomass (SB), Root Biomass (RB), Total plant biomass (PB), Grain carbon stocks ( $GC_S$ ), Shoot carbon stocks ( $SC_S$ ), Root carbon stocks ( $RC_S$ ) or Total plant carbon stocks (PCs) in  $\text{g m}^{-2}$

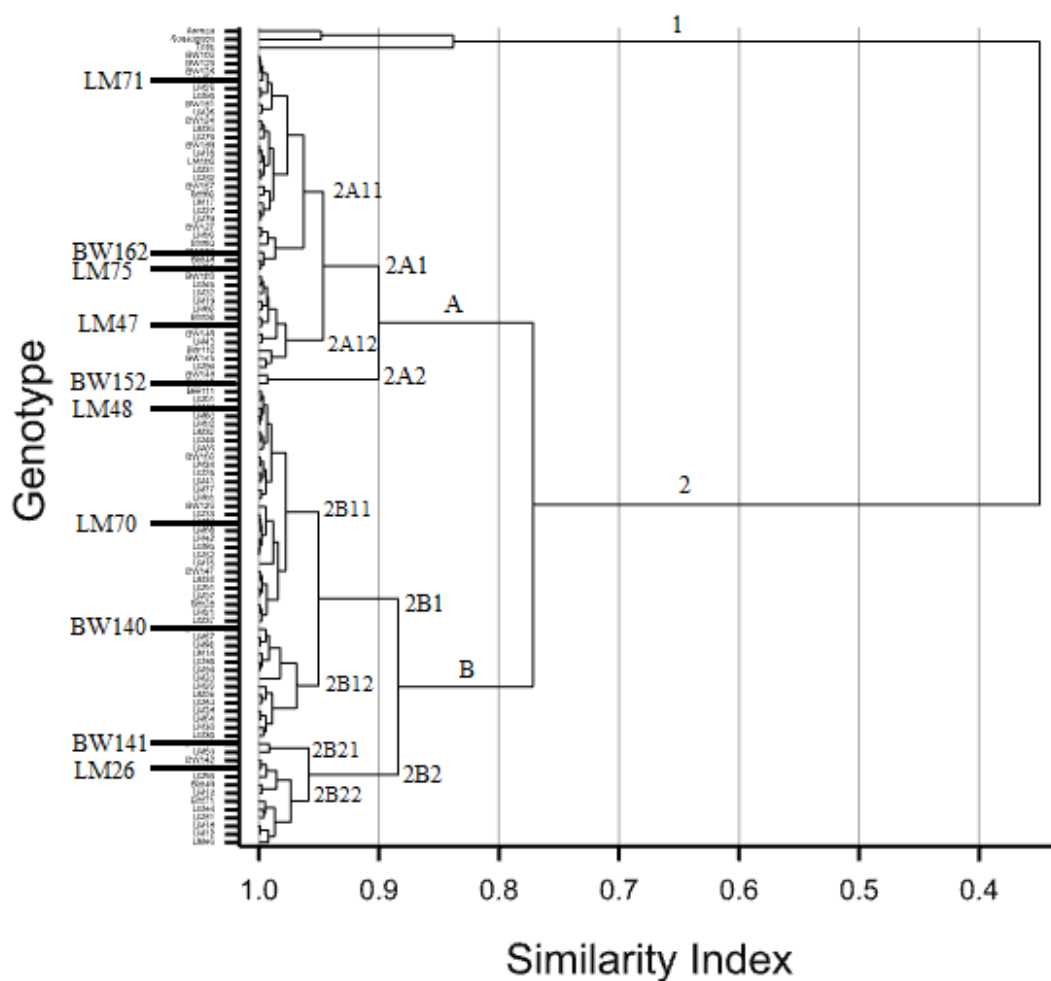
And **V** is the volume of irrigation water applied for the entire season in mm

The WUE data was normalised through averaging WUE of wheat genotypes from the field with those from the glasshouse, which resulted in average WUE of common genotype across both environments (field and glasshouse).

#### **4.3.4 Data analysis**

Analysis of variance (ANOVA) was conducted using the lattice procedure with GenStat 18<sup>th</sup> edition (Payne et al., 2017). In addition, the means of genotypes and the different water regimes were separated using least significant difference (LSD) at 0.05 significance level to quantify the effects of genotype, environment and water regime on measured variables. A multivariate

procedure for hierarchical clustering was performed based on phenotypic data combined across water regimes and sites, in order to group the genotypes according to similarity. A dendrogram was derived from a Euclidean similarity matrix using the unweighted pair group method with arithmetic mean algorithm (UPGMA) (Figure 4.1). Genotypes with high grain yield and biomass production in each cluster as indicated in Figure 4.1 were selected (to capture high performance and as much diversity as possible) for further development in the glasshouse.



**Figure 4.1:** Dendrogram showing clusters according to phenotypic relatedness of 100 genotypes evaluated.

## 4.4 Results

### 4.4.1 Variations in agronomic traits amongst wheat genotypes

The average grain yield (GY) amongst the initial set of 100 genotypes was 1387 g m<sup>-2</sup> with a standard error of  $\pm 84$  g m<sup>-2</sup> (Table 4.2). GY varied by a factor of 62 fold from 75 to 4696 g m<sup>-2</sup> in the field and showed a positively skewed distribution (Skew=1.53, Table 4.2). It was also significantly higher under non-stressed than water stressed conditions ( $p < 0.001$ , Appendix A.1; Table 4.3). However, total plant biomass (PB) was much less variable than GY as values ranged from 1976 to 13529 g m<sup>-2</sup>, a 6.8 times difference (Table 4.2), but was also higher under non-stressed compared to stressed conditions ( $p < 0.001$ , Appendix B.1). PB varied amongst the 100 wheat genotypes ( $p < 0.001$ , Appendix B), as it was lowest in genotype BW142 (1976 g m<sup>-2</sup>) and highest in genotype LM75 (6525 g m<sup>-2</sup>) under stressed conditions, while it was lowest in genotype LM32 (3509 g m<sup>-2</sup>) and highest in LM26 (13529 g m<sup>-2</sup>) under non-stressed conditions. R: S also varied amongst the 100 wheat genotypes ( $p < 0.001$ , Appendix C.1), with an average ratio of 0.12, meaning that they allocated about 12% of their total biomass to their roots, (ranges 3 to 38%, Table 4.2). Amongst the 100 wheat genotypes, LM75 exhibited the lowest (0.03), while genotype Tritic exhibited the highest R: S (0.38).

The interaction of GY as well as that of PB with water regimes and genotypes for the selected best 10 wheat genotypes in the field was significant ( $p < 0.05$ , Appendix D.1 and E.1). The sub-set of selected 10 genotypes also showed high variations in grain yield, plant total biomass and biomass allocation with for instance GY from 621 to 4383 g m<sup>-2</sup> (a 7.0-fold increase), PB with values between 2475 and 13529 g m<sup>-2</sup> (5.4 fold increase) (Table 4.2). Amongst the sub-set of selected 10 genotypes, LM71 exhibited the lowest GY (621 g m<sup>-2</sup>), while LM75 (3031 g m<sup>-2</sup>) exhibited the highest GY under stressed conditions (Table 4.2). On the other hand, BW140 (1624 g m<sup>-2</sup>) resulted in lower grain yield, while BW141 (4383 g m<sup>-2</sup>) resulted in higher grain

yield under non-stressed conditions. In this sub-set, PB was lowest in genotype LM47 (2475 g m<sup>-2</sup>) and highest in genotype LM75 (6525 g m<sup>-2</sup>) under stressed conditions; while under non-stressed conditions, it was lowest in genotype BW162 (4971 g m<sup>-2</sup>) and highest in genotype LM26 (13529 g m<sup>-2</sup>) (Table 4.2).

**Table 4.2:** Summary statistics for grain yield (GY), selected morphological variables SB: shoot biomass, RB: Root Biomass, PB: Total Plant Biomass and root to shoot ratio: R: S for the 100 and best 10 selected wheat genotypes grown in the field and across water regimes.

	<b>GY</b>	<b>SB</b>	<b>RB</b>	<b>PB</b>	<b>R:S</b>
	-----g m <sup>-2</sup> -----				
<i><b>100 genotypes</b></i>					
<i>(field)</i>					
Mean	1387	2498	305	4189	0,12
Median	1309	2332	263	3930	0,11
Min	75	1179	65	1976	0,03
Max	4696	8658	1219	13529	0,38
Q1	959	1827	189	3026	0,09
Q3	1644	2908	365	4893	0,15
CV%	47	37	57	37	41
SEM	84	121	22	200	0,01
Skew	1,53	2,04	2,03	1,82	1,67
Kurt	4,35	7,15	5,76	5,66	4,39
<i><b>Best 10 selected genotypes</b></i>					
<i>(field)</i>					
Mean	1882	3169	356	5408	0,12
Median	1624	2929	326	5214	0,10
Min	621	1598	152	2475	0,06
Max	4383	8658	1006	13529	0,26
Q1	1349	2317	249	3952	0,09
Q3	2500	3691	427	6140	0,13
CV%	45	44	47	42	35
SEM	109	182	22	290	0,01
Skew	1,05	2,03	1,91	1,65	1,60
Kurt	1,03	6,08	5,89	4,19	3,40

#### ***4.4.2 Variations in the wheat genotype's ability to store carbon***

The average carbon content in the grains, shoots and roots was not affected by watering regime ( $p > 0.05$ , Appendices F.1, G.1 & H.1), but significantly differed amongst the wheat genotypes ( $p < 0.05$ , Appendices F.1, G.1 & H.1). The carbon content in the grains, roots and shoots did not differ much under glasshouse and field conditions. It was on average  $40 \pm 0.96\%$  in grains ( $GC_C$ ),  $34 \pm 0.9\%$  in the shoots ( $SC_C$ ) and  $30 \pm 1.1\%$  in the roots ( $RC_C$ ) (Table 4.3).  $SC_C$  ranged from 30 to 37%, while  $RC_C$  varied from 21 to 39%, the later corresponding to a 90% increase (Table 4.3). Amongst the 10 selected wheat genotypes BW141 exhibited the lowest while LM70 exhibited the highest  $GC_C$ , and LM26 exhibited the lowest while LM48 exhibited the highest  $RC_C$ . Lastly LM75 exhibited the lowest with LM70 exhibiting the highest  $SC_C$ . However, the carbon stocks in the shoots, roots and total plant biomass showed greater variations. For instance, the total plant carbon stocks ( $PC_S$ ) exhibited a mean of  $996 \pm 31 \text{ g m}^{-2}$  with values ranging from 691 to  $1315 \text{ g m}^{-2}$  and corresponding to 2 times (i.e. 200%) difference under stressed conditions in the glasshouse and averaged  $2058 \pm 78 \text{ g m}^{-2}$  with values ranging from 1380 to  $3093 \text{ g m}^{-2}$  under non-stressed conditions (Table 4.3). In the field the  $PC_S$  had an average of  $1361 \pm 46 \text{ g m}^{-2}$  with values ranging from 835 to  $1913 \text{ g m}^{-2}$  under stressed conditions, while under non-stressed conditions the average  $PC_S$  were  $2287 \pm 107$  with values ranging from 1395 to  $4016 \text{ g m}^{-2}$  (Table 4.3).

#### ***4.4.3 Variation of agronomic traits and the wheat genotype's ability to store carbon as influenced by water regimes and the environment***

The interaction of grain yield (GY) with watering regime and genotypes in both the field and the glasshouse was significant ( $p < 0.05$ , Appendix D.1 & I.1). The field conditions were more conducive than glasshouse conditions for grain yield production since the average grain yield



produced were 1700 and 326 g m<sup>-2</sup> for field and glasshouse, respectively, under stressed conditions (Table 4.3). GY ranged from 58 to 869 g m<sup>-2</sup> with a standard error of  $\pm 28$  under stressed conditions in the glasshouse, whilst it ranged from 621 to 4488 g m<sup>-2</sup> with a standard error of  $\pm 116$  under stressed conditions in the field (Table 4.3). Under stressed conditions in the field GY was lowest in genotype LM71 (621 g m<sup>-2</sup>) and highest in LM75 (4488 g m<sup>-2</sup>). While under non-stressed conditions in the field, GY was lowest in genotype BW140 (1118 g m<sup>-2</sup>) and highest in BW141 (4383 g m<sup>-2</sup>). In the glasshouse under stressed conditions BW162 (58 g m<sup>-2</sup>) produced the lowest and LM75 produced the highest GY (869 g m<sup>-2</sup>) (Table 4.3). While under non-stressed conditions BW141 (136 g m<sup>-2</sup>) produced the lowest with BW162 yielding the highest GY (811 g m<sup>-2</sup>) (Table 4.3).

The interaction between total plant biomass (PB) with watering regime and genotypes was significant under both field and glasshouse ( $p < 0.001$ , Appendix E.1 and J.1). PB ranged from 4531 to 9305 g m<sup>-2</sup> under non-stressed conditions in the glasshouse, whilst it ranged from 3555 to 13529 g m<sup>-2</sup> in the field (Table 4.3). Under stressed conditions in the glasshouse BW152 (2156 g m<sup>-2</sup>) had the lowest PB, LM75 had the highest PB (3983 g m<sup>-2</sup>). While under non-stressed conditions LM26 (4531 g m<sup>-2</sup>) exhibited the lowest PB with BW140 exhibiting the highest PB (9305 g m<sup>-2</sup>). In the field under stressed conditions LM47 (2475 g m<sup>-2</sup>) exhibited the lowest PB, while LM75 exhibited the highest PB (8100 g m<sup>-2</sup>). Under non-stressed conditions BW162 (3555 g m<sup>-2</sup>) exhibited the lowest PB, while LM26 (13529 g m<sup>-2</sup>) exhibited the highest PB (Table 4.3). The interaction between total plant carbon stocks (PC<sub>s</sub>) with watering regime and 10 wheat genotypes in the glasshouse was significant ( $p < 0.001$ , Appendix AA.1). The PC<sub>s</sub> in the glasshouse under stressed conditions were highest (1315 g C m<sup>-2</sup>) in genotype LM75 and lowest (691 g C m<sup>-2</sup>) in BW152, while under non-stressed conditions PC<sub>s</sub> were highest (3093 g C m<sup>-2</sup>) in genotype BW140 and lowest (1380 g C m<sup>-2</sup>) in LM26 (Table

4.3). Watering regime significantly affected the total plant carbon stocks ( $PC_S$ ) of the selected 10 wheat genotypes under field conditions ( $p < 0.05$ , Appendix AB.1). Under stressed conditions in the field  $PC_S$  were highest ( $1913 \text{ g C m}^{-2}$ ) for genotype BW152 while they were lowest ( $835 \text{ g C m}^{-2}$ ) for LM70. Under non-stressed conditions the maximum  $PC_S$  was  $4016 \text{ g C m}^{-2}$  for genotype BW141 while the minimum  $PC_S$  was  $1395 \text{ g C m}^{-2}$  for BW140 (Table 4.3).

**Table 4.3:** Summary statistics for grain yield (GY), selected morphological variables (SB: shoot biomass, RB: Root Biomass, PB: Total Plant Biomass), and plant carbon variables (GCc: grain carbon content SCc: shoot carbon content, RCc: Root carbon content, GCs: Grain carbon stocks, SCs: Shoot carbon stocks, RCs: Root carbon stocks and PCs: Plant carbon stocks) for selected 10 wheat varieties grown in the field and glasshouse and across water regimes.

	GY	SB	RB	PB	R:S	GCC	SCC	RCC	GCs	SCs	RCs	PCs
	-----g m <sup>-2</sup> -----					-----%-----			-----g C m <sup>-2</sup> -----			
<b>Glasshouse</b>												
<i>25% Field capacity</i>												
<b>Mean</b>	326	1873	792	2992	0,43	40	33	31	132	625	239	996
<b>Median</b>	266	1826	762	2918	0,44	40	34	31	104	621	225	1012
<b>Min</b>	58	1349	368	2156	0,15	37	25	21	23	409	103	691
<b>Max</b>	869	2415	1263	3983	0,7	44	37	39	365	805	379	1315
<b>CV%</b>	68	24	36	24	34	18	19	23	69	25	34	24
<b>SEM</b>	28	57	37	94	0,02	0,96	0,84	0,89	11,77	19,85	10,48	30,63
<i>75% Field capacity</i>												
<b>Mean</b>	414	3726	2149	6289	0,57	40	33	31	167	1243	647	2058
<b>Median</b>	397	3440	1823	5508	0,57	40	34	31	150	1186	605	1814
<b>Min</b>	136	2779	1290	4531	0,37	37	25	21	55	851	333	1380
<b>Max</b>	811	5192	3575	9305	0,78	44	37	39	332	1822	1059	3093
<b>CV%</b>	50	26	38	30	26	18	19	23	52	27	35	29
<b>SEM</b>	27	127	104	247	0,02	0,96	0,84	0,89	11,22	44,05	29,18	77,87
<b>Field</b>												
<i>25% Field capacity</i>												
<b>Mean</b>	1700	2507	302	4508	0,12	40	34	30	439	833	89	1361
<b>Median</b>	1401	2534	277	4042	0,12	40	34	30	415	845	76	1385
<b>Min</b>	621	1598	152	2475	0,08	38	30	21	175	534	45	835
<b>Max</b>	3031	3775	642	6525	0,19	44	37	39	904	1215	214	1913
<b>CV%</b>	53	27	39	34	24	4	6	15	42	28	49	26
<b>SEM</b>	116	87	15	199	0,004	0,23	0,27	0,58	24,06	29,70	5,65	45,87
<i>75% Field capacity</i>												
<b>Mean</b>	2062	3714	403	6179	0,12	40	34	30	830	1331	126	2287
<b>Median</b>	1720	3154	382	5462	0,1	40	34	30	714	1211	117	2076
<b>Min</b>	1624	1954	135	4971	0,06	38	30	21	393	811	63	1395
<b>Max</b>	4383	8658	1006	13529	0,26	44	37	37	1843	2869	302	4016
<b>CV%</b>	44	42	44	39	43	5	7	14	49	40	44	36
<b>SEM</b>	117	202	23	313	0,01	0,24	0,29	0,54	52,19	69,50	7,08	107,18

#### ***4.4.4 Water use efficiency for grain, biomass production and carbon storage of wheat genotypes as influenced by the environment***

The interaction between WUE-GY with watering regime was significant in the glasshouse ( $p < 0.001$ , Appendix K.1), while it was not significant in the field, with only the watering regimes having a significant effect on WUE-GY in the field ( $p = 0.001$ , Appendix L.1). The average WUE for GY was  $0.74 \text{ g m}^{-2} \text{ mm}^{-1}$  in the glasshouse under stressed conditions, with values ranging from  $0.12$  to  $2.1 \text{ g m}^{-2} \text{ mm}^{-1}$  for BW162 and LM75 respectively (Table 4.4). In the glasshouse and under non-stressed conditions WUE for GY ranged from  $0.12$  to  $0.65 \text{ g m}^{-2} \text{ mm}^{-1}$  for BW141 and BW162 respectively with an average of  $0.33 \pm 0.02$  standard error. Higher WUE for GY was observed in the field, since the average was  $1.44$  and  $0.62 \text{ g m}^{-2} \text{ mm}^{-1}$  under stressed and non-stressed conditions respectively which was higher than WUE of GY in the glasshouse (Table 4.4).

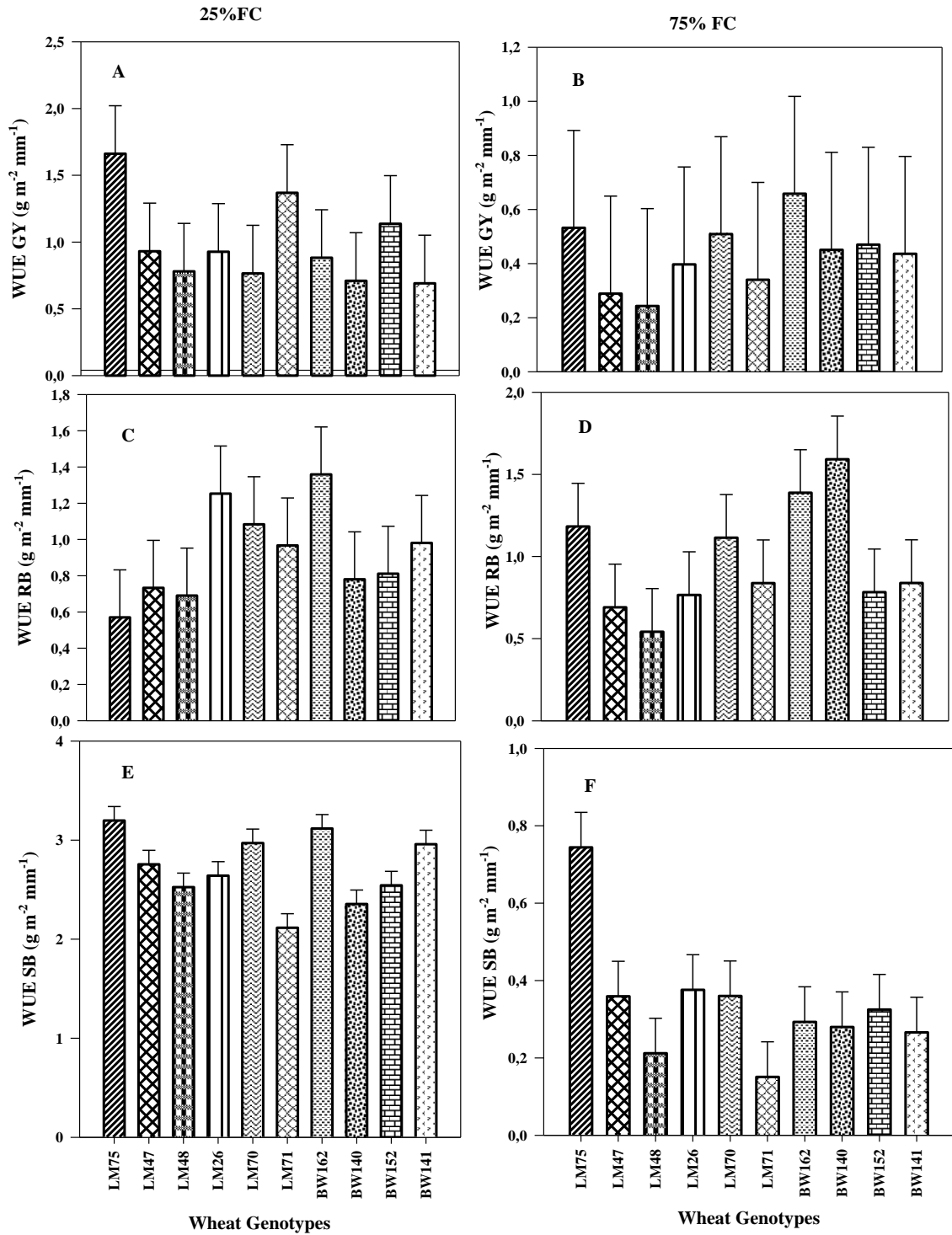
The WUE for total plant biomass (PB) had an average of  $6.58 \text{ g m}^{-2} \text{ mm}^{-1}$  and  $5.07 \text{ g m}^{-2} \text{ mm}^{-1}$  in the glasshouse under stressed and non-stressed conditions respectively (Table 4.4). Watering regime significantly affected WUE-PB in the field ( $p < 0.001$ , Appendix M.1). It ranged from  $2.19$  to  $7.24 \text{ g m}^{-2} \text{ mm}^{-1}$  for LM26 and BW152 respectively under stressed conditions, and from  $1.09 \text{ g m}^{-2} \text{ mm}^{-1}$  to  $3.9 \text{ g m}^{-2} \text{ mm}^{-1}$  for LM47 and BW141 respectively under non-stressed conditions. The WUE of total plant carbon stocks (WUE-PCs) ranged from  $1.16$  to  $2.68 \text{ g m}^{-2} \text{ mm}^{-1}$  with an average of  $2.12 \text{ g m}^{-2} \text{ mm}^{-1}$  in the glasshouse under stressed conditions (Table 4.4; significant at  $p < 0.001$  for all treatments, Appendix N.1). While in the field it had an average of  $1.12 \text{ g m}^{-2} \text{ mm}^{-1}$  and  $0.65 \text{ g m}^{-2} \text{ mm}^{-1}$  under stressed and non-stressed conditions respectively (Table 4.4).

**Table 4.4:** Summary statistics for Crop WUE (WUE-GY: Grain yield WUE, WUE-SB: Shoot Biomass WUE, WUE-RB: Root biomass WUE, WUE-PB: Plant biomass WUE), and Plant carbon WUE (WUE GCs: Grain carbon stocks WUE, WUE SCs: Shoot carbon stocks WUE; WUE-RCs: Root carbon stock WUE and WUE-PCs: Total plant carbon stocks WUE) for the 10 selected wheat varieties grown in the field and glasshouse and across water regimes (stressed and non-stressed).

	<b>GY</b>	<b>SB</b>	<b>RB</b>	<b>PB</b>	<b>GCs</b>	<b>SCs</b>	<b>RCs</b>	<b>PCs</b>
-----g m <sup>-2</sup> mm <sup>-1</sup> -----								
<b>Glasshouse</b>								
<i>Stressed conditions</i>								
<b>Mean</b>	0.74	4.10	1.74	6.58	0,23	1.37	0.52	2.12
<b>Median</b>	0.55	4.16	1.71	6.49	0,18	1.38	0.53	1.76
<b>Min</b>	0.12	2.63	0.89	4.21	0,04	0.80	0.25	1.16
<b>Max</b>	2.10	5.84	3.06	9.63	0,64	1.95	0.77	2.68
<b>CV</b>	75	20	34	22	69	20	28	17
<b>SEM</b>	0.07	0.10	0.08	0.19	0,02	0.04	0.02	0.04
<i>Non-stressed</i>								
<b>Mean</b>	0.33	3.00	1.74	5.07	0,12	1.00	0.52	2.64
<b>Median</b>	0.31	2.73	1.58	4.51	0,11	0.94	0.49	1.31
<b>Min</b>	0.12	2.28	0.94	3.56	0,04	0.64	0.30	1.10
<b>Max</b>	0.65	4.42	3.04	7.92	0,24	1.55	0.90	2.55
<b>CV</b>	51	26	39	31	52	27	36	29
<b>SEM</b>	0.02	0.10	0.09	0.20	0,01	0.04	0.02	0.06
<b>Field</b>								
<i>Stressed conditions</i>								
<b>Mean</b>	1.44	2.11	0.25	3.80	0,34	0.70	0.08	1.12
<b>Median</b>	1.25	2.00	0.23	3.37	0,32	0.69	0.07	1.07
<b>Min</b>	0.57	1.35	0.13	2.19	0,14	0.42	0.04	0.65
<b>Max</b>	4.01	3.49	0.55	7.24	0,70	1.12	0.18	1.45
<b>CV%</b>	55	28	40	36	42	27	48	28
<b>SEM</b>	0.10	0.08	0.01	0.18	0,02	0.02	0.00	0.03
<i>Non-stressed</i>								
<b>Mean</b>	0.62	1.11	0.12	1.84	0,24	0.37	0.04	0.65
<b>Median</b>	0.53	0.94	0.11	1.69	0,20	0.32	0.03	0.59
<b>Min</b>	0.33	0.60	0.04	1.09	0,11	0.21	0.02	0.39
<b>Max</b>	1.26	2.50	0.30	3.90	0,53	0.83	0.09	1.15
<b>CV%</b>	43	40	44	37	49	40	43	37
<b>SEM</b>	0.03	0.06	0.01	0.09	0,01	0.02	0.00	0.02

#### ***4.4.5 Water use efficiency for grain yield and biomass production across the field and the glasshouse as influenced by water regimes***

WUE components were significantly affected by water regime and differed amongst the wheat genotypes ( $p < 0.05$ , Appendices T.1, U.1 and V.1). On average the WUE for grain yield (GY) was much higher under stressed conditions than under non-stressed conditions, with all the stressed conditions values above  $0.75 \text{ g m}^{-2} \text{ mm}^{-1}$  and non-stressed conditions values below  $0.75 \text{ g m}^{-2} \text{ mm}^{-1}$  (Figure 4.2). Under stressed conditions the two best genotypes were LM75 with  $1.66 \text{ g m}^{-2} \text{ mm}^{-1}$  followed by LM71 with  $1.37 \text{ g m}^{-2} \text{ mm}^{-1}$ . BW141 and BW140 had the lowest WUE for GY with values of  $0.69$  and  $0.71 \text{ g m}^{-2} \text{ mm}^{-1}$  respectively which did not significantly differ with genotypes LM70 and LM48 (Figure 4.2A). This corresponded to a maximum 141% difference in WUE for GY under stressed conditions. Under non-stressed conditions, BW162 exhibited the highest WUE for GY amongst all the genotypes ( $0.66 \text{ g m}^{-2} \text{ mm}^{-1}$ ), whilst LM48 and LM47 had the lowest with average values of  $0.24 \text{ g m}^{-2} \text{ mm}^{-1}$  and  $0.29 \text{ g m}^{-2} \text{ mm}^{-1}$  respectively (Figure 4.2B). Irrespective of the water regime, LM75 was ranked first for GY WUE (Appendix AC.1).



**Figure 4. 2:** Wheat genotype and soil water availability (25 and 75 % field capacity) impacts on WUE for GY (A, B), RB (C, D) and SB (E, F). Bars are mean and error bars are LSD ( $p < 0.05$ ) of normalized data across field and glasshouse experiments.

The WUE for total plant biomass (WUE-PB) had an average of  $6.58 \text{ g m}^{-2}\text{mm}^{-1}$  and  $5.07 \text{ g m}^{-2} \text{ mm}^{-1}$  in the glasshouse under stressed and non-stressed conditions respectively. While in the field it ranged from 2.19 to  $7.24 \text{ g m}^{-2} \text{ mm}^{-1}$  and from  $1.09 \text{ g m}^{-2} \text{ mm}^{-1}$  to  $3.9 \text{ g m}^{-2} \text{ mm}^{-1}$  for stressed and non-stressed conditions respectively (Table 4.4). WUE for total plant biomass (PB) under stressed conditions was, as for GY the highest for LM75 ( $6.1 \text{ g m}^{-2} \text{ mm}^{-1}$ ) and the lowest for LM48 ( $4.2 \text{ g m}^{-2} \text{ mm}^{-1}$ ) was obtained which corresponded to a 45% difference (Figure 4.4A). On the other hand, BW140 had higher WUE-PB ( $4.58 \text{ g m}^{-2} \text{ mm}^{-1}$ ) and LM47 had lower ( $1.56 \text{ g m}^{-2} \text{ mm}^{-1}$ ) under non-stressed conditions (Figure 4.4B). LM75 was also ranked first for PB WUE (Appendix AC.1).

#### ***4.4.6 Water use efficiency for carbon storage across the field and glasshouse as influenced by water regimes***

Analysis of variance showed that WUE for GC<sub>s</sub>, SC<sub>s</sub>, RC<sub>s</sub> and PC<sub>s</sub> significantly differed between watering regimes and wheat genotypes ( $p < 0.05$ ) (Appendix O.1, P.1, Q.1 and R.1). WUE for plant carbon stocks varied within the plant parts (Figure 4.3) and were affected by the environment and amount of water applied (Table 4.4). WUE-GC<sub>s</sub> ranged from 0.04 to  $0.64 \text{ g C m}^{-2} \text{ mm}^{-1}$  a 16-fold increase in the glasshouse under stressed conditions whilst it ranged from 0.04 to  $0.24 \text{ g C m}^{-2} \text{ mm}^{-1}$  a 6-fold increase under non-stressed conditions (Table 4.4). The average WUE-PC<sub>s</sub> was  $2.12 \pm 0.04 \text{ g C m}^{-2} \text{ mm}^{-1}$  with values ranging from 1.16 to  $2.68 \text{ g C m}^{-2} \text{ mm}^{-1}$  under stressed conditions in the glasshouse, while under non-stressed conditions the average WUE-PC<sub>s</sub> was  $2.64 \pm 0.06 \text{ g C m}^{-2} \text{ mm}^{-1}$  with values ranging from 1.1 to  $2.55 \text{ g C m}^{-2} \text{ mm}^{-1}$  (Table 4.4). In the field the WUE-PC<sub>s</sub> had an average of  $1.12 \pm 0.03 \text{ g C m}^{-2} \text{ mm}^{-1}$  with values ranging from 0.65 to  $1.45 \text{ g C m}^{-2} \text{ mm}^{-1}$  under stressed conditions. While under non-stressed conditions the average WUE-PC<sub>s</sub> was  $0.65 \pm 0.02 \text{ g C m}^{-2} \text{ mm}^{-1}$  with values ranging from 0.39 to  $1.15 \text{ g C m}^{-2} \text{ mm}^{-1}$  (Table 4.4).



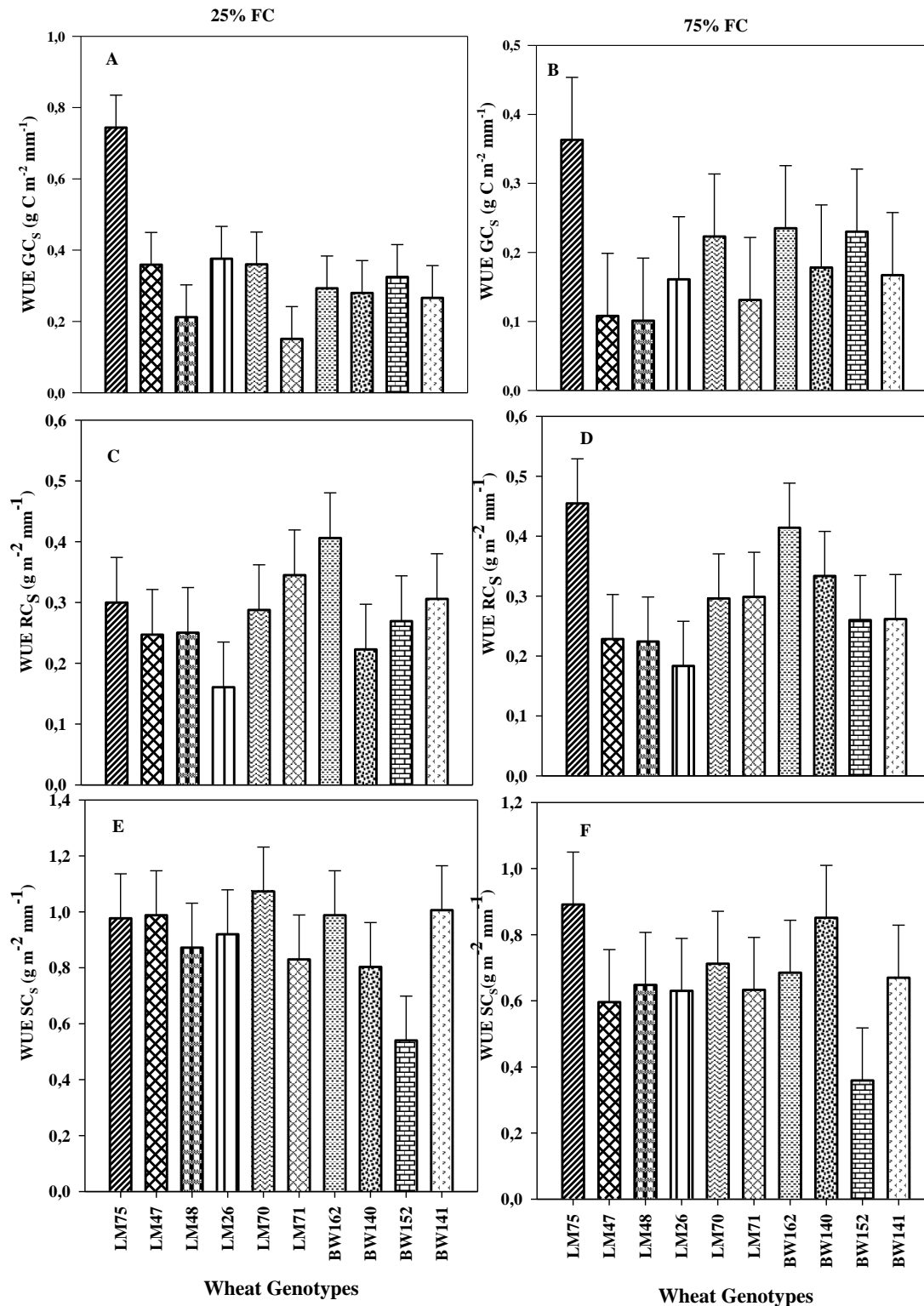
In general, the average WUE carbon stocks were higher under stressed (25%FC) than non-stressed conditions (75%FC) except for WUE RC<sub>s</sub> and WUE-RB (Table 4.5). For example, average WUE-GC<sub>s</sub> under stressed conditions was 0.337 g C m<sup>-2</sup> mm<sup>-1</sup> while under non-stressed conditions it was 0.19 g C m<sup>-2</sup> mm<sup>-1</sup> a 77% difference (Table 4.5). The average of WUE-SC<sub>s</sub> was 0.9 g C m<sup>-2</sup> mm<sup>-1</sup> under stressed conditions while it was 0.668 g C m<sup>-2</sup> mm<sup>-1</sup> under 75%FC a 35% difference (Table 4.5).

**Table 4.5:** Average of parameters as influenced by different water regimes

Parameters	25%FC	75%FC
GC <sub>s</sub> g m <sup>-2</sup>	327.63	497.95
RC <sub>s</sub> g m <sup>-2</sup>	181.06	436.48
SC <sub>s</sub> g m <sup>-2</sup>	698.77	1229.82
PC <sub>s</sub> g m <sup>-2</sup>	1203.66	2158.22
GY g m <sup>-2</sup>	810.79	1234.71
RB g m <sup>-2</sup>	596.13	1450.36
SB g m <sup>-2</sup>	2156.53	3721.23
PB g m <sup>-2</sup>	3601.45	6162.31
WUE-GC <sub>s</sub> g m <sup>-2</sup> mm <sup>-1</sup>	0.337	0.190
WUE-RC <sub>s</sub> g m <sup>-2</sup> mm <sup>-1</sup>	0.2794	0.2954
WUE-SC <sub>s</sub> g m <sup>-2</sup> mm <sup>-1</sup>	0.900	0.668
WUE-PC <sub>s</sub> g m <sup>-2</sup> mm <sup>-1</sup>	1.337	1.153
WUE-GY g m <sup>-2</sup> mm <sup>-1</sup>	0.896	0.423
WUE-RB g m <sup>-2</sup> mm <sup>-1</sup>	0.923	0.981
WUE-SB g m <sup>-2</sup> mm <sup>-1</sup>	0.904	0.672
WUE-PB g m <sup>-2</sup> mm <sup>-1</sup>	4.536	3.394

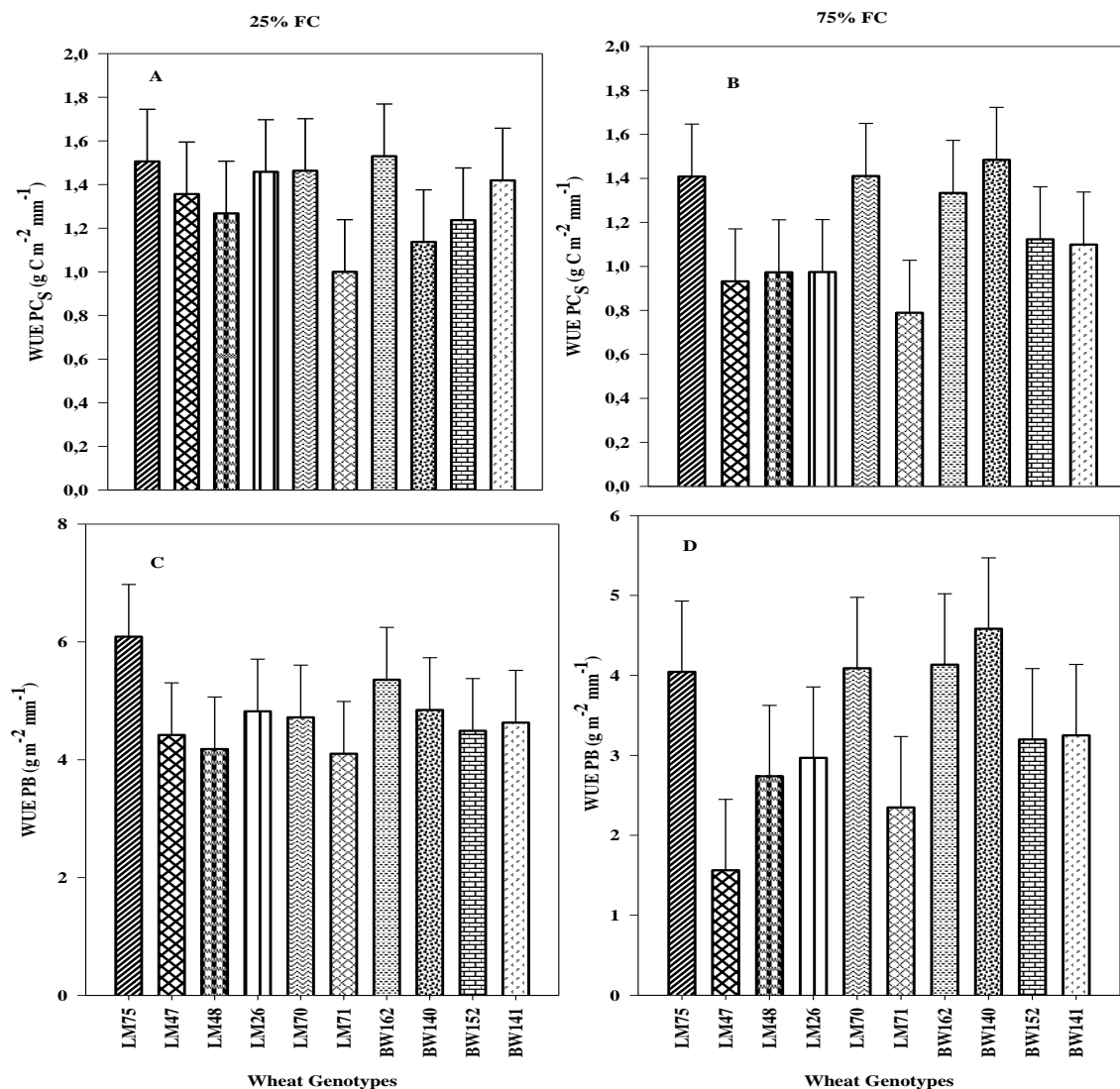
WUE-GC<sub>s</sub> under stressed conditions ranged from 0.151 g C m<sup>-2</sup> mm<sup>-1</sup> to 0.744 g C m<sup>-2</sup> mm<sup>-1</sup> for LM71 and LM75 respectively i.e. a 393% difference (Figure 4.3A). In contrast WUE-GC<sub>s</sub> under non-stressed conditions ranged from 0.101 g C m<sup>-2</sup> mm<sup>-1</sup> for LM48 to 0.363 g C m<sup>-2</sup> mm<sup>-1</sup> for LM75 which corresponds to a 259% difference (Figure 4.3B). Under stressed conditions BW162 exhibited higher WUE-RC<sub>s</sub> (0.406 g C m<sup>-2</sup> mm<sup>-1</sup>) with LM26 exhibiting lower WUE-RC<sub>s</sub> (0.16 g C m<sup>-2</sup> mm<sup>-1</sup>) (Figure 4.3C). While under non-stressed conditions LM75 had higher WUE-RC<sub>s</sub> (0.455 g C m<sup>-2</sup> mm<sup>-1</sup>) with LM26 exhibiting lower WUE-RC<sub>s</sub> (0.183 g C m<sup>-2</sup> mm<sup>-1</sup>).

<sup>1</sup>) (Figure 4.3D). WUE-SC<sub>s</sub> was generally higher than WUE-RC<sub>s</sub> both under stressed and non-stressed conditions. Amongst the 10 wheat genotypes LM70 exhibited higher WUE-SC<sub>s</sub> (1.073 g C m<sup>-2</sup> mm<sup>-1</sup>) with BW152 exhibiting lower WUE-SC<sub>s</sub> under stressed conditions (Figure 4.3E). Also, under non-stressed conditions LM75 showed higher WUE-SC<sub>s</sub> (0.891 g C m<sup>-2</sup> mm<sup>-1</sup>) while BW152 showed lower WUE-SC<sub>s</sub> (0.359 g C m<sup>-2</sup> mm<sup>-1</sup>) (Figure 4.3F).



**Figure 4.3:** Wheat genotype and soil water availability (25 and 75 % field capacity) impacts on WUE for GC<sub>s</sub> (A, B), RC<sub>s</sub> (C, D) and SC<sub>s</sub> (E, F). Bars are mean and error bars are LSD ( $p < 0.05$ ) of normalized data across field and glasshouse experiments.

The WUE for total plant carbon stocks (WUE-PC<sub>S</sub>) was highest in genotypes BW162 and LM75 with values of 1.53 and 1.51 g C m<sup>-2</sup> mm<sup>-1</sup> respectively and lowest in LM71 (1 g C m<sup>-2</sup> mm<sup>-1</sup>) under stressed conditions (Figure 4.4A). In contrast BW140 exhibited the highest WUE-PC<sub>S</sub> (1.484 g C m<sup>-2</sup> mm<sup>-1</sup>) with LM71 exhibiting the lowest WUE for PC<sub>S</sub> (0.789 g C m<sup>-2</sup> mm<sup>-1</sup>) under non-stressed conditions (Figure 4.4B).



**Figure 4. 4:** Wheat genotype and soil water availability (25 and 75 % field capacity) impacts on WUE for PC<sub>S</sub> (A, B) and PB (C, D). Bars are Mean and error bars LSD (p<0.05) of normalized data across field and glasshouse experiments.

#### ***4.4.7 Associations among agronomic traits as well as water use efficiency components across water regimes and the environments.***

There were generally high and significant correlations for most of the genetic traits analysed (Table 4.6). Positive significant correlations were observed between GY and PB ( $r=0.501$ ),  $PC_S$  ( $r=0.533$ ),  $SC_S$  ( $r=0.339$ ), WUE- $GC_S$  ( $r=0.769$ ), and WUE-GY ( $r=0.767$ ,  $p<0.001$ ). PB also positively correlated with  $RC_S$  ( $r=0.295$ ), SB ( $r=0.916$ )  $SC_S$  ( $r=0.894$ ,  $p<0.001$ ), RB ( $r=0.309$ ) and  $PC_S$  ( $r = 0.987$ ,  $p < 0.001$ ); while root biomass (RB) correlated positively with  $RC_S$  ( $r=0.985$ ), R:S ( $r=0.903$ ), SB ( $r=0.368$ ),  $SC_S$  ( $r=0.347$ ),  $PC_S$  ( $r=0.24$ ), WUE-PB ( $r=0.560$ ), WUE- $PC_S$  ( $r=0.665$ ), WUE-RB ( $r=0.861$ ), WUE- $RC_S$  ( $r=0.847$ ), WUE-SB ( $r=0.542$ ), and WUE- $SC_S$  ( $r=0.533$ ). Positive correlations were also between  $PC_S$  with  $RC_S$  ( $r=0.231$ ), SB ( $r=0.920$ ), and  $SC_S$  ( $r=0.913$ ,  $p<0.001$ ); while  $RC_S$  correlated positively with R:S ( $r=0.895$ ,  $p<0.001$ ), SB ( $r=0.346$ ),  $SC_S$  ( $r=0.325$ ), WUE-PB ( $r=0.545$ ), WUE- $PC_S$  ( $r=0.650$ ), WUE-RB ( $r=0.847$ ), WUE- $RC_S$  ( $r=0.866$ ), WUE-SB ( $r=0.527$ ), WUE- $SC_S$  ( $r=0.528$ ). The root to shoot ratio (R: S) had positive correlations with most of the WUE parameters such as WUE-PB ( $r=0.570$ ), WUE- $PC_S$  ( $r=0.644$ ), WUE-RB ( $r=0.910$ ,  $p<0.001$ ), WUE- $RC_S$  ( $r=0.901$ ), WUE-SB ( $r=0.524$ ), WUE- $SC_S$  ( $r=0.527$ ,  $p<0.001$ ); while SB correlated positively with  $SC_S$  ( $r=0.977$ ,  $p<0.001$ ) and WUE- $GC_S$  with WUE-GY ( $r=0.996$ ,  $p<0.001$ ).

The WUE parameters also has significant correlations among themselves, as WUE-PB had positive significant correlations with WUE- $PC_S$  ( $r=0.931$ ,  $p<0.001$ ), WUE-RB ( $r=0.811$ ), WUE- $RC_S$  ( $r=0.776$ ), WUE-SB ( $r=0.958$ ,  $p<0.001$ ) and WUE- $SC_S$  ( $r=0.902$ ,  $p<0.001$ ); while WUE- $PC_S$  had positive correlations with WUE-RB ( $r=0.841$ ), WUE- $RC_S$  ( $r=0.817$ ), WUE-SB ( $r=0.941$ ,  $p<0.001$ ) and WUE- $SC_S$  ( $r=0.911$ ,  $p<0.001$ ). On the other hand WUE-RB had positive correlations with WUE- $RC_S$  ( $r=0.973$ ,  $p<0.001$ ), WUE-SB ( $r=0.783$ ,  $p<0.001$ ) and WUE- $SC_S$  ( $r=0.771$ ); while WUE- $RC_S$  correlated positively with WUE-SB ( $r=0.756$ ) and

WUE-SC<sub>s</sub> (r=0.732); and WUE-SB had positive correlations with WUE-SC<sub>s</sub> (r=0.966, p<0.001) (Table 4.6).

Negative significant correlations also existed among the traits (Table 4.6) e.g. between GY with RC<sub>s</sub> (r=-0.590), RB (r=-0.579), WUE-PB (r=-0.365), WUE-PC<sub>s</sub> (r=-0.503), WUE-RB (r=-0.658), WUE-RC<sub>s</sub> (r=-0.681), WUE-SB (r=-0.484), and WUE-SC<sub>s</sub> (r=-0.508, p<0.001). While RB correlated negatively with WUE-GY (r=-0.563) and WUE-GC<sub>s</sub> (r=-0.545); and RC<sub>s</sub> also had negative correlations with WUE-GY (r=-0.583) and WUE-GC<sub>s</sub> (r=-0.569, p<0.001). The R: S negatively correlated with WUE-GC<sub>s</sub> (r=-0.539) and WUE-GY (-0.545). Again, negative significant correlations were observed between WUE-GC<sub>s</sub> and WUE-RB (r=-0.349) as well as WUE-RC<sub>s</sub> (r=-0.394, p<0.001), while WUE-GY negatively correlated with WUE-RB (r=-0.365) and WUE-RB (r=-0.403)

**Table 4. 6:** Pearson’s coefficients (r) showing correlations of agronomic traits and WUE components of wheat genotypes across environments

	<b>GY</b>	<b>PB</b>	<b>PC<sub>s</sub></b>	<b>RB</b>	<b>RCC</b>	<b>RC<sub>s</sub></b>	<b>R_S</b>	<b>SB</b>	<b>SCC</b>	<b>SC<sub>s</sub></b>	<b>WUE_GC<sub>s</sub></b>	<b>WUE_GY</b>	<b>WUE_PB</b>	<b>WUE_PC<sub>s</sub></b>	<b>WUE_RB</b>	<b>WUE_RC<sub>s</sub></b>	<b>WUE_SB</b>	<b>WUE_SC<sub>s</sub></b>	
<b>GY</b>	1.000																		
<b>PB</b>	0.501**	1.000																	
<b>PC<sub>s</sub></b>	0.553**	0.987**	1.000																
<b>RB</b>	-0.579	0.309*	0.240*	1.000															
<b>RCC</b>	-0.131	-0.144	-0.101	-0.068	1.000														
<b>RC<sub>s</sub></b>	-0.590**	0.295*	0.231*	0.985**	0.079	1.000													
<b>R_S</b>	-0.695	-0.008	-0.087	0.903**	-0.062	0.895**	1.000												
<b>SB</b>	0.332	0.916**	0.920**	0.368**	-0.181	0.346**	0.018	1.000											
<b>SCC</b>	-0.029	-0.059	0.014	-0.019	-0.093	-0.048	-0.071	0.047**	1.000										
<b>SC<sub>s</sub></b>	0.339**	0.894**	0.913**	0.347**	-0.169	0.325**	-0.003	0.977	0.191	1.000									
<b>WUE_GC<sub>s</sub></b>	0.769**	0.139	0.176	-0.545**	-0.213	-0.569**	-0.539**	-0.013	-0.027	-0.019	1.000								
<b>WUE_GY</b>	0.767**	0.116	0.154	-0.563**	-0.187	-0.583**	-0.545**	-0.040	-0.051	-0.045	0.996**	1.000							
<b>WUE_PB</b>	-0.365**	0.017	-0.049	0.560**	-0.167	0.545**	0.570**	0.002	-0.109	-0.040	0.094	0.079	1.000						
<b>WUE_PC<sub>s</sub></b>	-0.503**	-0.027	-0.082	0.665**	-0.109	0.650**	0.644**	0.016	-0.022	-0.012	-0.086	-0.101	0.931**	1.000					
<b>WUE_RB</b>	-0.658**	0.002	-0.077	0.861**	-0.144	0.847**	0.910**	0.040	-0.044	0.013	-0.349**	-0.365**	0.811**	0.841**	1.000				
<b>WUE_RC<sub>s</sub></b>	-0.681**	-0.021	-0.090	0.847**	0.044	0.866**	0.901**	0.020	-0.105	-0.013	-0.394**	-0.403**	0.776**	0.817**	0.973**	1.000			
<b>WUE_SB</b>	-0.484**	-0.090	-0.142	0.542**	-0.145	0.527**	0.524**	-0.014	-0.048	-0.050	-0.018	-0.034	0.958**	0.941**	0.783**	0.756**	1.000		
<b>WUE_SC<sub>s</sub></b>	-0.508**	-0.110	-0.143	0.553**	-0.175	0.528**	0.527**	0.018	0.139	0.015	-0.047	-0.065	0.902**	0.911**	0.771**	0.732**	0.966**	1.000	

Significant at  $p < 0.05 = *$ , Significant at  $p < 0.01 = **$ , GY: Grain yield, PB: Total plant biomass, PCS: Total plant carbon stocks, RB: Root biomass, RCC: Root carbon content, RCS: Root carbon stocks, R:S-Root to shoot ratio, SB: Shoot biomass, SCC: Shoot carbon content, SCS: Shoot carbon stocks, WUE-GY: Grain yield WUE, WUE-SB: Shoot Biomass WUE, WUE-RB: Root biomass WUE, WUE-PB: Plant biomass WUE, WUE GC<sub>s</sub>: Grain carbon stocks WUE, WUE SC<sub>s</sub>: Shoot carbon stocks WUE; WUE-RC<sub>s</sub>: Root carbon stock WUE and WUE-PC<sub>s</sub>: Total plant carbon stocks WUE

## 4.5 Discussion

### *4.5.1 Variations of crop agronomic traits with water regimes and the environment*

The agronomic traits (GY, RB, SB & PB) were significantly affected by watering regime ( $p < 0.05$ , Appendices O.1, P.1, Q.1 and R.1). They were generally lower under water stressed conditions and higher under well-watered conditions (Table 4.5). This was because moisture stress is known to reduce biomass and grain yield at any growth stage when it occurs (Akram 2011). Also, under water stressed conditions, hastened onset of senescence occurs which decreases photosynthesis leading to lower grain and biomass yield (Al-Ghzawi et al., 2018). This results in the crop not being able to capture solar radiation for biomass production and it also shortens the time frame for mobilization and translocation of N assimilates from plant tissues to the grain (Grogan et al., 2016). The lower grain yield under water stress could also be due to the limited water availability at booting stage which has been reported to reduce grain yield production (Al-Ghzawi et al., 2018). Similar results were reported by Saadi et al., (2015) who found a significant reduction in grain yield under stressed conditions due to the reduction of photo-assimilates production for grain filling.

Under moisture stress, the plant also struggles to absorb photo-assimilates thereby reducing grain filling duration. It has been reported that water stress during the reproductive development stages decreased grain and biomass yield (Ciadir et al., 1999). Furthermore, Al-Ghzawi et al., (2018) reported an increase in grain yield when wheat cultivars were irrigated to 100% FC. The increase in grain and biomass yield due to improved water availability has also been demonstrated in many studies (Hussain et al., 2004; Kang et al., 2001; Wajid et al., 2004). Biomass allocation between roots and shoots, expressed as R: S ratios also increased at higher water regime in the glasshouse (Table 4.3). Similar results were reported by Othmani et al.,



(2015), who reported increase of R: S of durum wheat with increasing amount of irrigation water.

The environment in which the wheat cultivars were grown also had a significant effect on wheat agronomic traits with GY in the field ( $1700 \text{ g m}^{-2}$ ) being significantly higher than in the glasshouse ( $326 \text{ g m}^{-2}$ , Table 4.3). Lower wheat grain yields under glasshouse conditions have been reported by Anwar et al., (2007); Sharma et al., (2008); Farooq et al., (2011); and may be due to the fact that in the greenhouse, temperature may exceed the optimal  $21^{\circ}\text{C}$ , which is likely to result in reduced wheat grain yields. It is necessary for wheat to experience chilling temperatures at the early stages of growth for optimal grain filling, which usually do not occur in the glasshouse.

#### ***4.5.2 Variation of water use efficiency for grain and biomass production between genotypes as influenced by water regimes and the environment***

Water use efficiency (WUE) was significantly affected by water regime ( $p < 0.05$ ) (Appendix S.1, T.1, U.1 and V.1). It was generally higher for most of the measured traits under stressed than non-stressed conditions in both the glasshouse and field (Table 4.4). This is in accordance with the findings by Chibarabada et al., (2015) who reported higher WUE of Bambara groundnut under severely stressed (25% FC) compared to non-stressed (75% FC) conditions. Abbate et al., (2004) also reported an increase of WUE of wheat with limited water supply. This was attributed to reduction of stomatal aperture and transpiration rate of wheat under stressed conditions (Pirasteh-Anosheh et al., 2016). Higher WUE under stressed conditions could also be attributed to that plant roots extract more soil water from a greater depth under conditions of moisture stress compared to when they are irrigated (El Hwary and Yagoub, 2011). This means that stored soil water under water stress can be used more efficiently.

WUE of wheat genotypes varied under both stressed and non-stressed conditions ( $p < 0.05$ ). Similar results were reported by Balouchi (2010) who observed significant variation of WUE between cultivars. LM75 had highest WUE for GY under stressed conditions, whilst LM48 exhibited lower WUE for GY under stressed conditions (Figure 4.2A). A similar trend was observed for WUE-PB under stressed conditions (Figure 4.4C). These results reveal that LM75 is not sensitive to water stress for grain and biomass production, whilst LM48 is sensitive and is therefore not the best genotype for grain and biomass production when water supply is limited. Under non-stressed, BW162 exhibited highest WUE for GY (Figure 4.2B) whilst BW140 exhibited higher WUE for PB (Figure 4.4D), but LM47 produced the least WUE for both GY and PB (Figure 4.2B and 4.4D). Therefore, from these findings it can be deduced that when water supply is sufficient, BW140 and BW162 could be desirable genotypes for optimal GY and biomass production. Michirio et al., (1994) pointed out that drought tolerant wheat cultivars will show increased while drought sensitive ones will show decreased WUE when water-stressed. Overall, LM75 was ranked the best genotype for WUE of both GY and PB, while BW162 was ranked the best for WUE of RB production (Appendix AC.1). Therefore, depending on the farmer's area of interest these cultivars can be used for wheat production under limited water availability.

#### ***4.5.3 Variation of wheat genotypes in WUE for carbon storage***

Annual plant C input into the soil is one of the major factors determining the quantity of organic matter in agro-ecosystems. Plant carbon vary between different crop species, cultivars and different plant parts of the same species (Bolinder et al., 1997). There was low variation in the carbon content between the roots (RCc) and shoots (SCc). RCc ranged from 21 to 39% whilst SCc ranged from 25 to 37% (Table 4.3). This lower variability in carbon content is accounted

for by the similar biophysical characteristics (microclimate and land management) in the study area (Salas Macías et al., 2017). However, carbon stocks in the grain (GCs), shoots (SCs), roots (RCs) and in total plant biomass (PCs) showed greater variation (Table 4.3). This proved that wheat genotypes can act as storehouses of carbon by stocking it in their tissues, thereby lowering the levels of atmospheric greenhouse gases as stated by Brown et al., (1989). The variation in carbon stocks led to variation of WUE for carbon stocks amongst the wheat genotypes.

Generally, WUE for most carbon stocks (WUE-GCs, WUE-SCs and WUE-PCs) except for WUE-RCs was higher ( $p < 0.05$ ) under water-stressed compared to non-stressed conditions (Table 4.5). This could be due to that under water stress, there is a decrease in stomatal conductance, and an increase in photosynthetic rate (Farquhar et al., 1980). Therefore, more carbon is assimilated and less water is transpired (Zhang et al., 2012). WUE-RCs was highest in BW162 under stressed conditions while it was highest in genotype LM75 under non-stressed conditions (Figure 4.3C-D), and the lowest WUE-RCs was observed in LM26 under both stressed and non-stressed conditions. This suggests that genotypes BW162 and LM75 are ideal genotypes for improving soil C. The root carbon and soil carbon pool have been reported to have direct relationship since most soil organic matter is derived from roots (Dietzel et al., 2017). Therefore, the higher WUE-RCs exhibited by genotype BW162 suggests that growing it will result in higher soil organic matter build-up thereby improving soil fertility under limited water availability. This also suggests that BW162 can produce below ground carbon stocks with lesser amount of water which aid in carbon sequestration. According to Lal (2004), carbon allocated below ground contributes to soil fertility and carbon sequestration.

The positive significant correlation observed between RCs with WUE-RCs and WUE-PCs (Table 4.6), suggests that an increase in RCs results in increased WUE-RCs and WUE-PCs

thus causing the wheat genotypes to be ideal for carbon sequestration. The WUE for shoot carbon stock (WUE-SC<sub>s</sub>) was highest in genotypes LM70 and BW141 under stressed conditions while under non-stressed conditions LM75 exhibited higher WUE-SC<sub>s</sub> (Figure 4.3E-F). The lowest WUE-SC<sub>s</sub> was observed in genotype BW152 both under stressed and non-stressed conditions (Figure 4.3E-F). These results suggest that LM70, BW141 and LM75 are ideal genotypes for production of shoot carbon stocks. From these findings it can also be argued that drought tolerant wheat cultivars will assimilate more carbon with less water. Above ground biomass has been reported to constitute an important visible carbon pool (Ravindranath and Ostwald, 2008). Genotype LM75 was ranked the best genotype for WUE-GC<sub>s</sub>, WUE-SC<sub>s</sub> and WUE-PC<sub>s</sub> (Appendix AC.1). This indicates that it has the highest potential for shoot and total plant carbon stocks production hence carbon sequestration potential. However, if its residues are not incorporated into the soil, the C stock could be lost into the atmosphere. Therefore, for optimal soil C sequestration, the biomass for LM75 should be incorporated into the soil after harvesting.

## 4.6 Conclusions

The results obtained in this study showed that wheat genotypes significantly differed in their response to scenarios of water availability. Under water-stressed conditions, genotypes LM75 exhibited highest while BW141 exhibited lowest WUE-GY. However, under non-stressed conditions BW162 exhibited highest, while LM48 and LM47 had the lowest WUE-GY. WUE-PB was also highest in genotype LM75 and lowest in LM71 and LM48 under stressed conditions, while under non-stressed conditions, it was highest in genotype BW140 and lowest in LM47. The different components of carbon stocks varied as well within the different wheat cultivars. WUE-RC<sub>s</sub> was highest in BW162 and lowest in genotype LM26 under both water-stressed and non-stressed conditions. While WUE-SC<sub>s</sub> was highest in genotypes LM70 and BW141 as well as lowest in genotype BW152 under stressed conditions. Whereas under non-stressed conditions, WUE-SC<sub>s</sub> was highest in LM75 and lowest in genotype BW152. On the other hand, WUE-PC<sub>s</sub> was highest in genotypes BW162 and LM75 and lowest in LM71 under stressed conditions, while under non-stressed conditions it was highest in BW140 with genotype LM71 exhibiting the lowest WUE-PC<sub>s</sub>.

When water availability is limited LM75 is an ideal genotype for grain and total biomass production of wheat since it is not sensitive to drought stress. On the other hand, the wheat genotype BW162 is an ideal candidate to build plant carbon stocks since it exhibited the highest WUE-PC<sub>s</sub> under water-stressed conditions. Therefore, these genotypes are recommended to wheat farmers for GY, PB and PC<sub>s</sub> production under water stressed conditions.

## CHAPTER FIVE

### MINERALIZATION PATTERNS AND SOIL CARBON SEQUESTRATION POTENTIAL OF WHEAT RESIDUES FROM DIFFERENT GENOTYPES

#### 5.1 Abstract

Sequestration of atmospheric carbon (C) into plants and ultimately to soils is one of the strategies to mitigate climate change and restore C reserves of degraded land. Incorporation of crop residues can therefore be done to improve soil productivity. As these residues decompose in soil, they release mineral nutrients such as carbon, nitrogen, phosphorus and sulphur among others. Wheat is an important food crop to the South African economy. It is produced intensively as an irrigated crop on commercial farms. Not much is known however about the potential of its residues to sequester soil C or release mineralizable nutrients once incorporated into the soil. The objective of this study was to assess soil C sequestration potential and mineralization patterns of wheat residues from different genotypes upon incorporation into the soil. About 0.25 g each of wheat root (RT) or shoot (ST) from genotypes LM70, LM75, BW140, BW152 and BW162 were thoroughly mixed with 100 g of soil then transferred into an air tight PVC pot. NaOH solution was also placed inside the incubation pot to trap CO<sub>2</sub> released during decomposition, and this was measured on day 0, 7, 15, 23, 31, 39, 47, 55, 63, 77, 91, 105, and 120 of incubation. Moist soil from each pot was also analysed for NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and extractable P mineralized during each incubation period using a Gallery Discrete Auto analyser (Scientific Thermo Fisher 2014). In general, the shoot treatments evolved higher net CO<sub>2</sub>-C and mineralized higher net mineral N and extractable P compared to root treatments. Net CO<sub>2</sub>-C evolution was highest within the first two weeks and declined with time. Amongst

the root treatments, BW140 RT evolved the highest net CO<sub>2</sub>-C (86.6 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil), while LM70 RT evolved the lowest (48.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil). In shoot treatments BW162 ST and BW140 ST evolved the highest net CO<sub>2</sub>-C with average values of 218.7 and 223.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil respectively. Comparing all the 10 treatments LM70 RT evolved the lowest while BW140 ST and BW162 ST had the highest net CO<sub>2</sub>-C. The net total N mineralization however increased with increasing incubation period. BW140 RT mineralized the highest net total N compared to all root treatments (34.50 mg N kg<sup>-1</sup> soil), while BW152 RT had the lowest (8.39 mg N kg<sup>-1</sup> soil). In the shoot treatments BW162 ST, BW140 ST and BW152 ST mineralized the highest net total N while LM70 ST and LM75 ST had the lowest. In all the 10 treatments, BW152 RT mineralized lower while BW140 ST, BW162 ST and BW152 ST had the highest net total N concentration. Not much increase in P mineralization was observed in the first 2 weeks of incubation. In the root treatments BW162 RT mineralized the highest net extractable P (2.14 mg P kg<sup>-1</sup> soil), while BW140 RT had the lowest (1.5 mg P kg<sup>-1</sup> soil). In the shoot treatments BW152 ST had the highest net extractable P (3.69 mg P kg<sup>-1</sup> soil), while BW140 ST mineralized the lowest (2.47 mg P kg<sup>-1</sup> soil). Comparing all the 10 treatments, BW152 ST mineralized the highest while BW140 RT had the lowest net extractable P. All in all, the highest N and P mineralization was observed in the shoots while more C sequestration was registered in root treatments since they released less net CO<sub>2</sub>-C.

**Key words:** wheat residues, N and P mineralization, carbon sequestration

## 5.2 Introduction

Declining soil quality is an increasing issue threatening crop production in South Africa (Mills and Fey, 2003). Soil organic carbon (SOC) is a key indicator of soil quality and it plays a vital role in nutrient cycling, improving soil physico-chemical and biological properties as well as crop production (Lal 2004). Increasing population, indiscriminate use of inorganic fertilizers (Feiziene and Kadžiene, 2008), intensive tillage and grazing, or frequent burning are major causes for soil deterioration in South Africa (Mills and Fey, 2003). The maintenance of SOC is crucial for sustainable agricultural production as declining soil C generally decreases crop productivity (Lal 2006). Strategies for improving soil C reserves include the application of organic materials (i.e. crop residues, green manure, animal manure) into the soil to enhance the SOC pool. The SOC pool in agricultural soils can enhance agricultural sustainability and serve as a potential sink for CO<sub>2</sub> (Gnanavelrajah et al. 2008). Soil C sequestration can improve soil quality and reduce contribution of agriculture to CO<sub>2</sub> emissions. IPCC, (1996) reported that CO<sub>2</sub> concentration in the atmosphere has been increasing at a rate of  $3.2 \times 10^{15}$  g C year<sup>-1</sup>, with 20% of this contributed by agriculture (Lal 2001). Therefore, to counteract this higher rate of CO<sub>2</sub> and other greenhouse gases emissions from the soil, organic soil amendments must be added to the soil.

The application of crop residues may reduce the emissions of CO<sub>2</sub>, whilst improving crop yields. Incorporation of crop residues is important for the maintenance of organic carbon (C) and N stocks in the nutrient pool of arable soils (Rasmussen and Parton, 1994). It provides readily available nutrients to soils for plant uptake depending upon the decomposition rates of residues and synchrony of nutrient mineralization (Lupwayi et al., 2005). Ayuke et al., (2004) reported an increase in mineral N content and maize yields when residues of *Tithonia diversifolia* were incorporated into soil compared with inorganic fertilizers. Oguike et al.



(2006) found that rice residues used as a soil amendment displayed relatively higher potentials for improving physico-chemical properties of nutrient depleted Haplic Acrisols compared to NPK fertilizer. Abro et al (2011) reported that CO<sub>2</sub> emission from the soil was reduced when maize straw was retained into the soil and SOC significantly increased. In South Africa wheat is a widely grown crop and is the second important cereal crop after maize (Gbetibono and Hassan, 2005). However, its potential to sequester C through reduction of CO<sub>2</sub> emissions from the soil, as well as its N and P mineralization potential is not well known. Therefore, the aim of the study was to compare mineralization patterns and soil carbon sequestration potential of wheat residues from different genotypes.

### **5.3 Materials and Methods**

The wheat residue incubation experiment was conducted in a laboratory at the University of Kwa-Zulu Natal, Pietermaritzburg Campus, South Africa from May- September 2018.

#### ***5.3.1 Soil Sampling***

Soil used in the incubation was collected from an arable field located at Ukulinga Research Farm of the University of Kwa-Zulu Natal, Kwa-Zulu Natal Province, South Africa. The soil in the study site was loam in texture classified as Chromic Luvisols (FAO, soil Classification). Soil samples were collected from a depth of 0-15 cm at random points using a soil auger, then mixed thoroughly to form a composite sample. The soil sample was air dried, ground and passed through a 2 mm sieve. A sub-sample of 0.5 kg was taken for determination of soil physical and chemical properties using standard soil analysis procedures and the results are presented in Table 5.1.

**Table 5.1:** Physico-chemical properties of the soil used in the incubation study.

<b>Soil Property</b>	<b>Values</b>
Bulk density (g cm <sup>-3</sup> )	1.24
Sand (%)	29.96
Silt (%)	34.87
Clay (%)	24.43
Texture	Loam
pH (KCl)	4.73
P (mg L <sup>-1</sup> )	11
K (mg L <sup>-1</sup> )	114
Ca (mg L <sup>-1</sup> )	1294
Mg (mg L <sup>-1</sup> )	389
Exch. Acidity (cmol L <sup>-1</sup> )	0.047
Zn (mg L <sup>-1</sup> )	3.6
Mn (mg L <sup>-1</sup> )	15
Cu (mg L <sup>-1</sup> )	7.2
TC %	1.9
TN%	0.17

### ***5.3.2 Collection, characterization and selection of wheat residues***

The residues of 10 wheat genotypes from a previous WUE study (chapter 4) were separated into roots and shoots, oven dried at 60°C for 72 hrs (as stated in chapter 4), then ground to pass through a 1 mm sieve before chemical analysis. Triplicate samples of plant residues were taken and analysed for total C, N, P and lignin concentrations. Total N and C were analysed using the LECO Trumac CNS auto analyser version 1.1x (LECO Corporation, 2012). The lignin content was determined using Van Soest methods (Van Soest et al., 1991). Total P was determined by digestion (sulphuric acid + hydrogen peroxide) using electric hot plate (Okalebo, 1993). Due to limited laboratory space, only five genotypes were selected for the incubation experiment based on their C: N ratios since these were more significantly different compared to their lignin contents. These included two genotypes with the lowest (BW162 and BW140),

two with the highest (BW152 and LM75) and one with moderate (LM70) C: N ratio. The biochemical properties of the selected genotypes are presented in Table 5.2.

**Table 5. 2:** Biochemical properties of selected wheat residues

Treatment	Genotype	Total C%	Total N%	Total P %	Lignin%	C: N	C: P	Lignin: N
Root	LM70	23.85 <sup>a</sup>	0.45 <sup>b</sup>	0.20 <sup>c</sup>	28.39 <sup>b</sup>	53.00 <sup>d</sup>	119.25 <sup>ab</sup>	63.09 <sup>f</sup>
Root	BW162	33.13 <sup>ab</sup>	0.64 <sup>c</sup>	0.21 <sup>d</sup>	29.32 <sup>b</sup>	51.77 <sup>d</sup>	157.76 <sup>f</sup>	45.81 <sup>e</sup>
Root	BW152	30.41 <sup>ab</sup>	0.34 <sup>a</sup>	0.16 <sup>b</sup>	25.51 <sup>b</sup>	89.44 <sup>g</sup>	190.6 <sup>g</sup>	75.03 <sup>g</sup>
Root	LM75	33.41 <sup>ab</sup>	0.41 <sup>ab</sup>	0.17 <sup>b</sup>	29.77 <sup>b</sup>	81.49 <sup>f</sup>	196.53 <sup>h</sup>	72.61 <sup>g</sup>
Root	BW140	28.19 <sup>ab</sup>	0.83 <sup>d</sup>	0.13 <sup>a</sup>	30.11 <sup>b</sup>	33.96 <sup>b</sup>	216.85 <sup>i</sup>	36.27 <sup>d</sup>
Shoot	LM70	34.03 <sup>b</sup>	0.65 <sup>c</sup>	0.26 <sup>ef</sup>	15.18 <sup>a</sup>	52.35 <sup>d</sup>	130.88 <sup>c</sup>	23.35 <sup>b</sup>
Shoot	BW162	31.59 <sup>b</sup>	1.60 <sup>e</sup>	0.27 <sup>f</sup>	14.16 <sup>a</sup>	19.74 <sup>a</sup>	117.00 <sup>ab</sup>	8.85 <sup>a</sup>
Shoot	BW152	34.51 <sup>b</sup>	0.88 <sup>d</sup>	0.35 <sup>h</sup>	10.72 <sup>a</sup>	39.22 <sup>c</sup>	98.6 <sup>a</sup>	12.18 <sup>a</sup>
Shoot	LM75	36.17 <sup>b</sup>	0.57 <sup>c</sup>	0.30 <sup>g</sup>	17.35 <sup>a</sup>	63.46 <sup>e</sup>	120.57 <sup>b</sup>	30.43 <sup>c</sup>
Shoot	BW140	34.86 <sup>b</sup>	1.64 <sup>e</sup>	0.25 <sup>e</sup>	16.26 <sup>a</sup>	21.26 <sup>a</sup>	139.44 <sup>d</sup>	9.91 <sup>a</sup>

RT=Root and ST=Shoot, Note: different letters in each column show significant differences among treatments at ( $p < 0.05$ )

### 5.3.3 Wheat residue incubation experiment

#### 5.3.3.1 CO<sub>2</sub>-emission determination

The incubation experiment was set up using a complete randomized design of 11 treatments replicated 3 times with 12 sampling times. The 11 treatments included the control (soil alone), as well as root (RT) or shoot (ST) treatments of five wheat genotypes namely LM70, BW152, LM75, BW162 and BW140. About 0.25 g of ground root or shoot of each genotype was mixed with 100 g of soil in a 100 ml PVC container, slowly wetted to fill up 50% pore space and placed in 500 ml airtight PVC pots. A vial containing 25 ml of NaOH solution was placed inside the PVC pot with soil to trap CO<sub>2</sub>. The pots were covered with polyethylene and incubated in the dark in a constant temperature room set at 25°C. The CO<sub>2</sub>-C evolved was measured at 0, 7, 15, 23, 31, 39, 47, 55, 63, 77, 91, 105 and 120th day after incubation, then

NaOH was titrated with 0.5M HCl after precipitating carbonates with BaCl<sub>2</sub>, using phenolphthalein as an indicator. The net CO<sub>2</sub>-C evolved was obtained by calculating differences in values of the biomass treated soil and control, while cumulative mineralised CO<sub>2</sub>-C was calculated as the sum of all previous measurements.

#### ***5.3.3.2 Mineral nitrogen and phosphorus determination***

After titrating for CO<sub>2</sub> emission, the incubated treatments were analysed for NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total mineral nitrogen (NH<sub>4</sub><sup>+</sup>-N +NO<sub>3</sub><sup>-</sup>) and extractable phosphorus. Triplicate samples from each treatment were removed from the PVC pots at different incubation times and extracted by shaking 2 g of the sample with 20 ml of 1M KCl for 1 hr followed by filtration. The concentration of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in the extract were analysed using Gallery Discrete Autoanalyzer (Scientific Thermo Fisher 2014). Extractable P was determined calorimetrically following Ambic-2 extraction and determined using Gallery Discrete Auto analyser. Net NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and extractable P were obtained by difference between values of the control and the biomass treated soil. The net mineralized N was calculated as the sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations released from that particular treatment after subtracting the control.

#### ***5.3.4 Statistical Analysis***

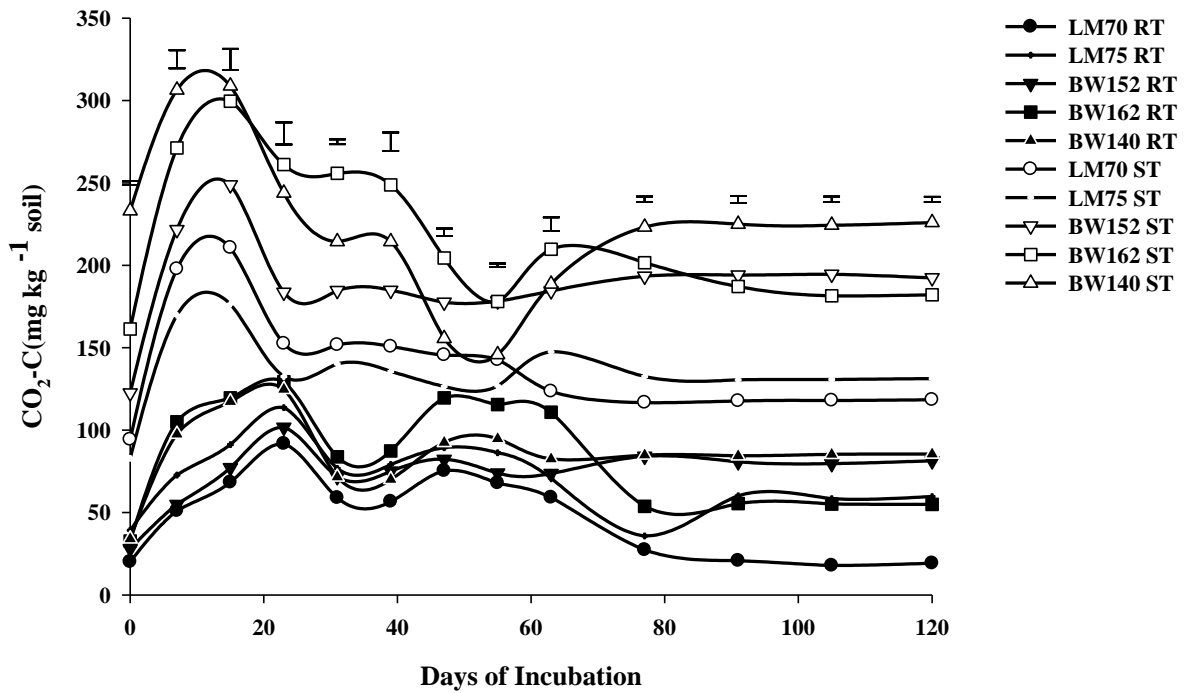
A two-way analysis of variance (ANOVA) was done on all parameters studied, the means of parameters were grouped together for comparisons and differences were separated by least significant differences (LSD) using GenStat 18<sup>th</sup> edition (Payne et al., 2017), at p = 0.05. The

bivariate correlations among CO<sub>2</sub>-C emissions, N and P mineralization and biochemical properties of wheat genotypes were done using the Pearson's rank correlations procedure.

## **5.4 Results**

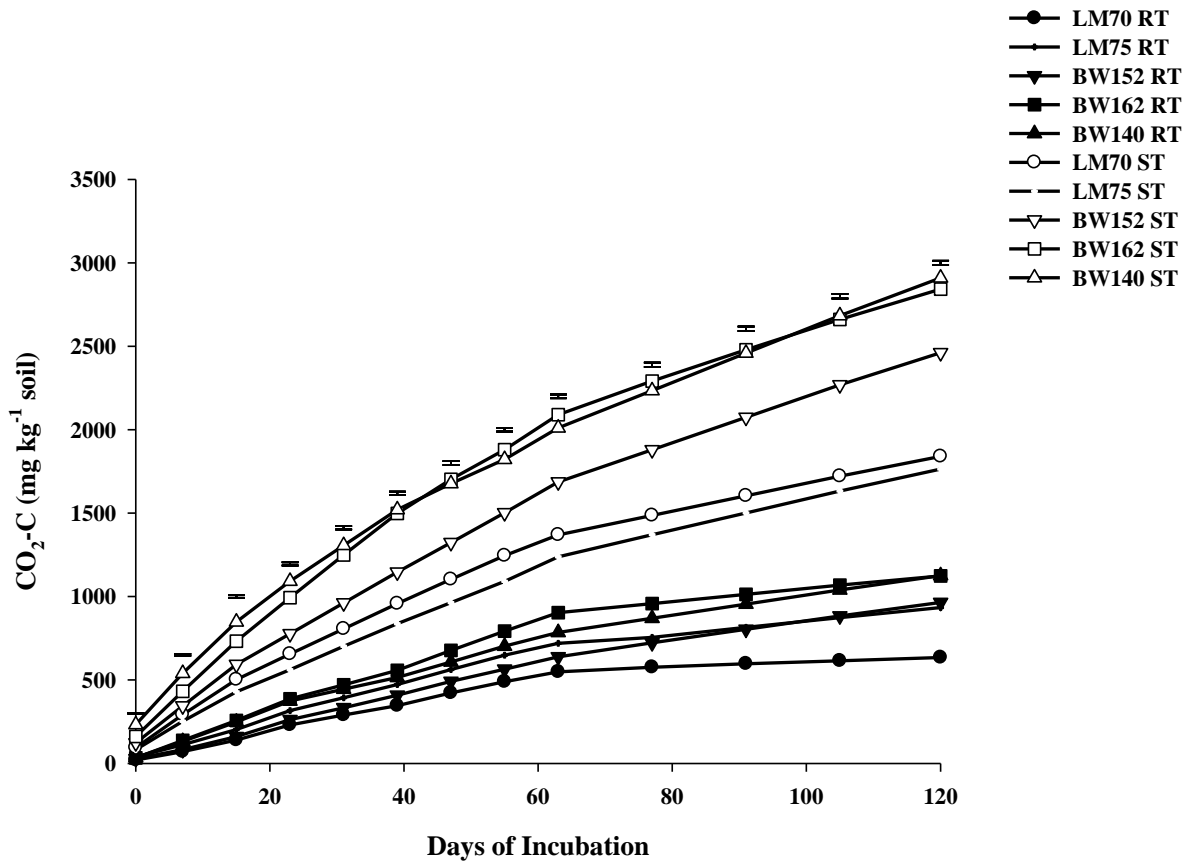
### ***5.4.1 Variation of CO<sub>2</sub> emission amongst different wheat residues***

The net CO<sub>2</sub>-C evolution significantly differed amongst all treatments and incubation times ( $p < 0.001$ , Appendix AD.1). It was high in the first 21 days of incubation and drastically decreased after day 21 reaching smaller peaks until stable CO<sub>2</sub> emissions were reached after day 77 of incubation (Figure 5.1). The shoot treatments evolved 147% higher net CO<sub>2</sub>-C than roots for all varieties (Tables 5.3 and 5.4). Amongst the root treatments, LM70 evolved the lowest net CO<sub>2</sub>-C with an average value of 48.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil, while the other four genotypes did not significantly differ (Table 5.4). In shoot treated soils, net CO<sub>2</sub>-C evolution increased from day 0, reaching maximum peaks on day 15 of incubation, with maximum net CO<sub>2</sub>-C values reached of 176.51, 210.65, 248.80, 299.56 and 308.82 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil for LM70, LM75, BW152, BW162 and BW140, respectively (Fig 5.1). In overall, BW162 ST and BW140 ST evolved higher net CO<sub>2</sub>-C with average values of 218.7 and 223.8 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil respectively, while LM70 ST and LM75 ST evolved the lowest net CO<sub>2</sub>-C with average values of 141.5 and 135.6 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil, respectively (Table 5.4). A comparison of all 10 treatments showed that LM70 RT evolved the lowest net CO<sub>2</sub>-C while BW140 ST and BW162 ST evolved highest (Table 5.4).



**Figure 5.1:** Net CO<sub>2</sub>-C emission from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 day period. Error bars represent the LSD ( $p < 0.001$ )

Net Cumulative CO<sub>2</sub>-C emission significantly differed amongst treatments ( $p < 0.001$ , Appendix AE.1). It increased with increasing incubation period, and LM70 RT (which did not significantly differ from LM75 RT and BW152 RT) exhibited the lowest cumulative net CO<sub>2</sub>-C with an average value of 383.29 mg CO<sub>2</sub>-C kg<sup>-1</sup>, while BW140 ST (as well as BW162 shoot) exhibited highest cumulative net CO<sub>2</sub>-C with an average value of 1641.43 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil (Table 5.4 and Figure 5.2).

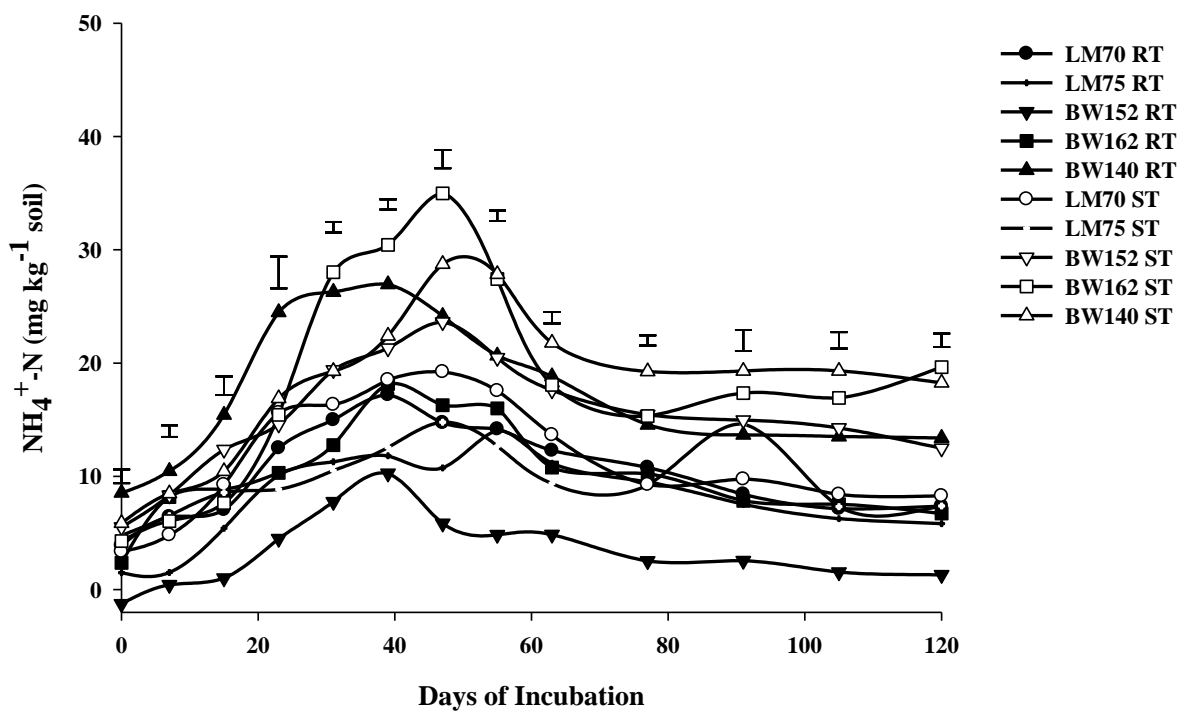


**Figure 5.2:** Net Cumulative CO<sub>2</sub>-C emission from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 day period. Error bars represent the LSD (p<0.001)

#### 5.4.2 Variation of N mineralization amongst different wheat varieties

Net NH<sub>4</sub><sup>+</sup>-N release significantly differed amongst all treatments and incubation days (p<0.001, Appendix AF.1). From the root treated soils, net NH<sub>4</sub><sup>+</sup>-N release increased from day 0 until maximum peaks were reached at day 39 of incubation (Figure 5.3). The maximum net NH<sub>4</sub><sup>+</sup>-N concentration released in root treated soils were 17.19, 14.05, 10.27, 18.15 and 26.95 mg N kg<sup>-1</sup> soil for LM70 RT, LM75 RT, BW152 RT, BW162 RT and BW140 RT, respectively. Amongst the root treatments BW140 RT released the highest net NH<sub>4</sub><sup>+</sup>-N (17.76 mg N kg<sup>-1</sup> soil), followed by LM70 RT and BW162 RT with average net NH<sub>4</sub><sup>+</sup>-N values of 10.53 and 10.47mg N kg<sup>-1</sup> soil, respectively while BW152 RT released the lowest net NH<sub>4</sub><sup>+</sup>-N with an average value of 3.56 mg N kg<sup>-1</sup> soil (Table 5.4). In the shoot treatments, BW162 ST, BW140

ST and BW152 ST released the highest net  $\text{NH}_4^+\text{-N}$  concentration with an average value of 18.58, 18.3 and 15.41  $\text{mg N kg}^{-1}$  soil while LM75 ST and LM70 ST released the lowest net  $\text{NH}_4^+\text{-N}$  concentration with average values of 9.78 and 11.84  $\text{mg N kg}^{-1}$  soil respectively (Table 5.4). A comparison of all the 10 treatments showed that both BW140 RT & ST, BW162 ST and BW152 ST released the highest net  $\text{NH}_4^+\text{-N}$  while BW152 RT released the lowest net  $\text{NH}_4^+\text{-N}$  (Table 5.4).

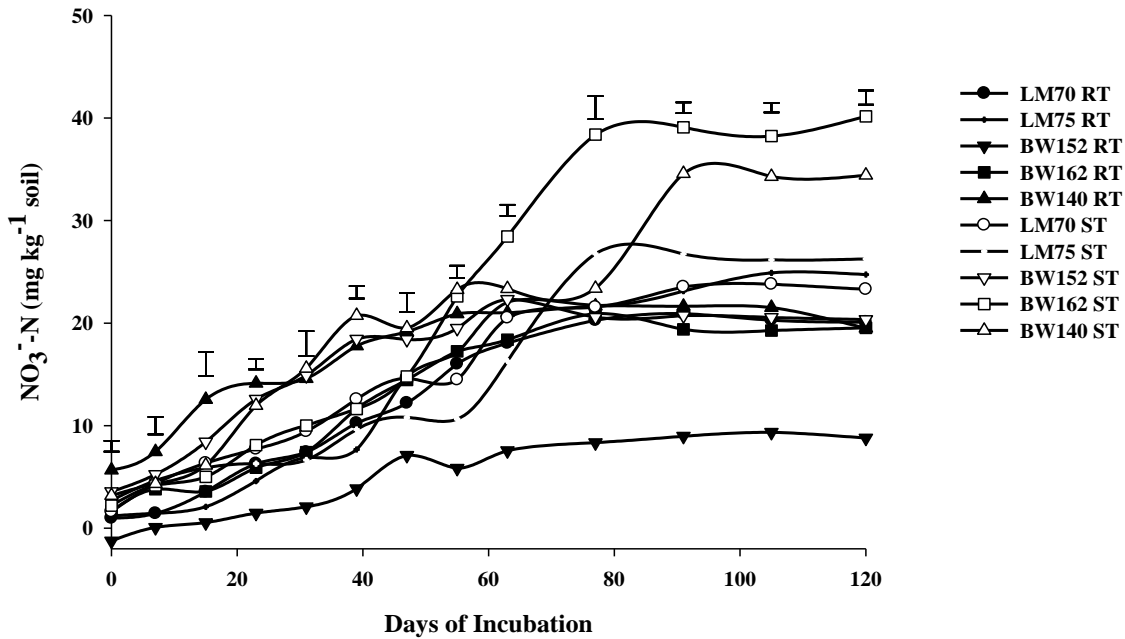


**Figure 5.3:** Net  $\text{NH}_4^+\text{-N}$  mineralized from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 day period. Error bars represent the LSD ( $p < 0.001$ )

Net  $\text{NO}_3^-\text{-N}$  concentration increased throughout the incubation period, with a rapid increase observed after day 55 of incubation (Figure 5.4). Amongst the root treatments BW152 RT released the lowest net  $\text{NO}_3^-\text{-N}$  concentration ( $4.83 \text{ mg N kg}^{-1}$  soil) while the other four treatments did not significantly differ (Table 5.4). In the shoot treatments BW162 ST and BW140 ST released the highest net  $\text{NO}_3^-\text{-N}$  with average concentrations of 20.22 and 19.6  $\text{mg N kg}^{-1}$  soil respectively (Table 5.4).



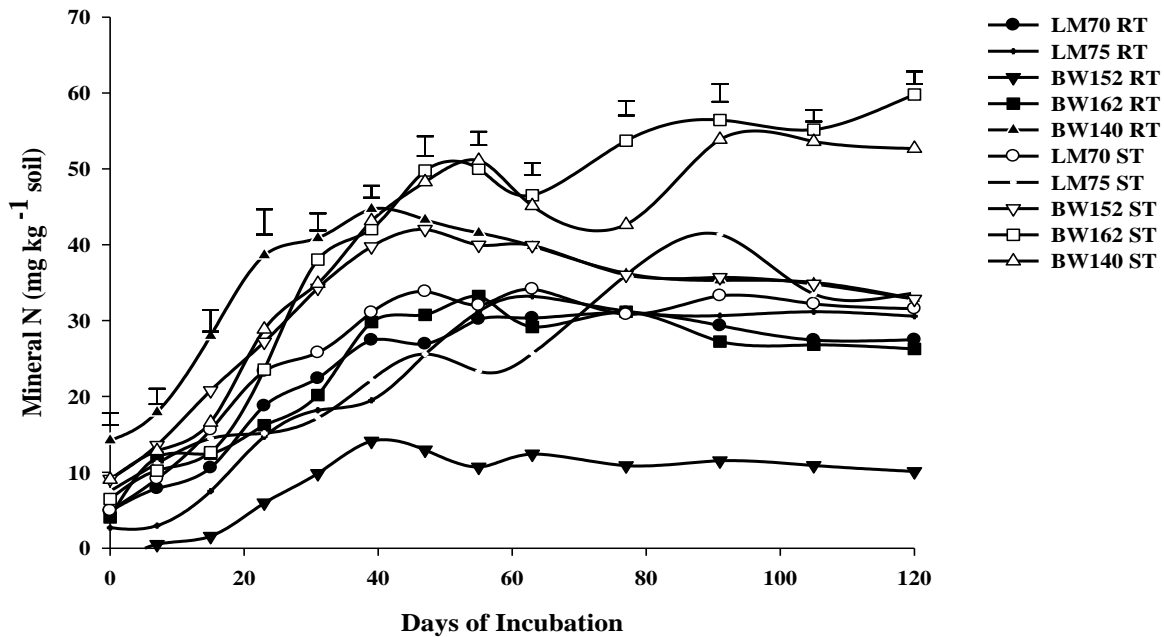
N kg<sup>-1</sup> soil respectively, while LM70 ST and LM75 ST released lowest net NO<sub>3</sub><sup>-</sup>-N with averages of 14.14 and 13.81 mg N kg<sup>-1</sup> soil. Comparing all the 10 treatments showed that BW162 ST, BW140 ST, BW152 ST, LM70 ST and BW140 RT released higher net NO<sub>3</sub><sup>-</sup>-N whilst BW152 RT released lowest concentration of net NO<sub>3</sub><sup>-</sup>-N (Table 5.4).



**Figure 5.4:** Net NO<sub>3</sub><sup>-</sup>-N mineralized from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 days period. Error bars represent the LSD (p<0.001)

The analysis of variance showed that net total mineralized N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) was significantly affected by all treatments (p<0.001, Appendix AH.1). It increased with increasing incubation period for most treatments (Figure 5.5). In general, the root residues mineralized lower net total N concentration (22.01 mg N kg<sup>-1</sup> soil) compared to shoot residues (31.5 mg N kg<sup>-1</sup> soil, Tables 5.3 and 5.4). Amongst the root treatments BW140 RT mineralized the highest net total N concentration with an average of 34.5 mg N kg<sup>-1</sup> soil while BW152 RT mineralized the lowest with an average value of 8.39 mg N kg<sup>-1</sup> soil. In the shoot treatments BW162 ST, BW140 ST and BW152 ST mineralized the highest net total N concentration with average N

concentration values of 38.8, 37.9 and 31.22 mg N kg<sup>-1</sup> soil, while LM70 ST and LM75 ST released lowest net total N concentration with average values of 25.98 mg N kg<sup>-1</sup> soil and 23.59 mg N kg<sup>-1</sup> soil, respectively (Table 5.4). Overall, BW152 RT mineralized the lowest net total N while BW140 ST, BW162 ST and BW140 RT released the highest net total N concentration.

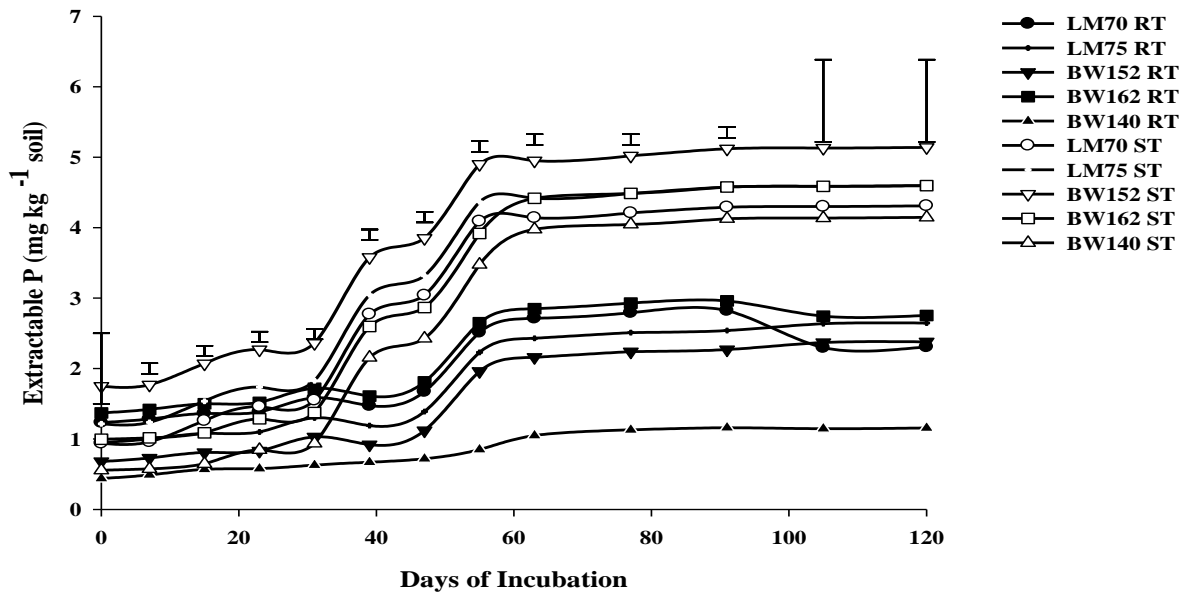


**Figure 5.5:** Net N mineralized (NH<sub>4</sub><sup>+</sup>-N +NO<sub>3</sub><sup>-</sup>-N) from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 day period. Error bars represent the LSD (p<0.001)

#### 5.4.3 Variation of P mineralization amongst different wheat residues

Net extractable P mineralization significantly differed amongst all treatments and incubation days (p<0.001, Appendix AI.1). Shoots released significantly higher net extractable P compared to root residues (p<0.001, Appendix AI.1) with average values of 1.64 and 3.02 mg P kg<sup>-1</sup> soil for roots and shoots respectively (Table 5.3). There was not much increase in net extractable P within the first few weeks of incubation (Figure 5.6). In root treatments a slow increase was observed from day 0, with an increase observed at day 55 of incubation until a stability was reached after day 91 of incubation (Figure 5.6). In root treatments BW162 RT

mineralized highest net extractable P followed by LM70 RT and LM75 RT with average values of 2.14, 1.96 and 1.77 mg P kg<sup>-1</sup> soil, respectively (Table 5.4). While BW140 RT released the lowest net extractable P (0.82 mg P kg<sup>-1</sup> soil) followed by BW152 RT with net extractable P average value of 1.5 mg P kg<sup>-1</sup> soil (Table 5.4).



**Figure 5.6:** Net P mineralized from roots (RT) and shoots (ST) of different wheat varieties incubated over a 120 day period. Error bars represent the LSD ( $p < 0.001$ )

In shoot treatments, a rapid increase was observed after day 31 of incubation until the stability was reached after day 63 of incubation (Figure 5.6). BW152 ST mineralized the highest net extractable P (3.69 mg P kg<sup>-1</sup> soil) followed by LM75 ST (3.15 mg P kg<sup>-1</sup> soil), while BW140 ST release the lowest net extractable P (2.47 mg P kg<sup>-1</sup> soil) (Table 5.4). Overall, BW152 ST, LM75 ST, BW162 ST mineralized the highest net extractable P while BW140 RT mineralized the lowest (Table 5.4).

**Table 5.3:** Average CO<sub>2</sub> emissions and mineralizable nutrients released from roots and shoots of wheat residues.

Parameter	Root	Shoot	LSD (p<0.001)
Net CO <sub>2</sub> -C (mg kg <sup>-1</sup> soil)	73.56	181.79	0.53
Net Cumulative CO <sub>2</sub> -C (mg kg <sup>-1</sup> soil)	529.02	1316.82	1.27
Net NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> soil)	10.11	14.78	0.09
Net NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> soil)	11.91	16.72	0.09
Net total mineralized N (mg kg <sup>-1</sup> soil)	22.01	31.49	0.13
Net mineral P (mg kg <sup>-1</sup> soil)	1.64	3.02	0.03

**Table 5.4:** Average net CO<sub>2</sub>-C emissions and mineralizable nutrients released from wheat residues of different genotypes

Treatment	Net CO <sub>2</sub> -C	Net Cum CO <sub>2</sub> -C	Net NH <sub>4</sub> <sup>+</sup> -N	Net NO <sub>3</sub> <sup>-</sup> -N	Net tot minN	Net min P
	-----mg kg <sup>-1</sup> soil-----					
LM70 RT	48.8 <sup>a</sup>	383.3 <sup>a</sup>	10.53 <sup>b</sup>	12.13 <sup>b</sup>	22.66 <sup>b</sup>	1.96 <sup>bc</sup>
LM75 RT	71.8 <sup>b</sup>	526.7 <sup>a</sup>	8.21 <sup>b</sup>	13.26 <sup>b</sup>	21.47 <sup>b</sup>	1.77 <sup>bc</sup>
BW152 RT	74.2 <sup>b</sup>	488.0 <sup>a</sup>	3.56 <sup>a</sup>	4.83 <sup>a</sup>	8.39 <sup>a</sup>	1.50 <sup>ab</sup>
BW162 RT	86.4 <sup>b</sup>	644.6 <sup>ab</sup>	10.47 <sup>b</sup>	12.56 <sup>b</sup>	23.02 <sup>b</sup>	2.14 <sup>bcd</sup>
BW140 RT	86.6 <sup>b</sup>	602.6 <sup>ab</sup>	17.76 <sup>d</sup>	16.74 <sup>bcd</sup>	34.50 <sup>d</sup>	0.82 <sup>a</sup>
LM70 ST	141.5 <sup>c</sup>	1052.1 <sup>c</sup>	11.84 <sup>bc</sup>	14.14 <sup>bcd</sup>	25.98 <sup>bc</sup>	2.87 <sup>de</sup>
LM75 ST	135.6 <sup>c</sup>	955.9 <sup>bc</sup>	9.78 <sup>b</sup>	13.81 <sup>bc</sup>	23.59 <sup>bc</sup>	3.15 <sup>ef</sup>
BW152 ST	189.3 <sup>d</sup>	1318.3 <sup>cd</sup>	15.41 <sup>cd</sup>	15.81 <sup>bcd</sup>	31.22 <sup>cd</sup>	3.69 <sup>f</sup>
BW162 ST	218.7 <sup>e</sup>	1616.4 <sup>d</sup>	18.58 <sup>d</sup>	20.22 <sup>d</sup>	38.80 <sup>d</sup>	2.91 <sup>def</sup>
BW140 ST	223.8 <sup>e</sup>	1641.4 <sup>d</sup>	18.30 <sup>d</sup>	19.60 <sup>cd</sup>	37.90 <sup>d</sup>	2.47 <sup>cde</sup>
<b>LSD</b>						
<b>(p&lt;0.001)</b>	13.37	240.3	2.384	3.836	5.056	0.5004

RT=Root and ST=Shoot, Net cum CO<sub>2</sub>-C= Net cumulative CO<sub>2</sub>-C, Net min N= Net total N mineralized, Net min P= Net mineralized P Note: different letters in each column show significant differences among treatments at (p < 0.001).

#### 5.4.4 Relationship between CO<sub>2</sub>-C emission, N and P mineralization with residue quality characteristics

Table 5.5 showed that significant positive correlation existed between total C and CO<sub>2</sub>-C (r= 0.61, p<0.05), NH<sub>4</sub><sup>+</sup>-N (r= 0.27, p<0.05), net total N mineralized (r= 0.25, p<0.05) as well as total P (r= 0.52, p<0.001). C: N correlated positively with C: P (r= 0.26, p<0.05), lignin (r= 0.37, p<0.05), lignin: N (r= 0.78, p<0.001) while C: P positively correlated with lignin (r= 0.15, p<0.05) and lignin: N (r=0.43, p<0.05). Lignin positively correlated with lignin: N (r=0.74,

$p < 0.001$ ) while  $\text{CO}_2\text{-C}$  correlated positively with  $\text{NH}_4^+\text{-N}$  ( $r = 0.35$ ,  $p < 0.05$ ),  $\text{NO}_3^-\text{-N}$  ( $r = 0.51$ ,  $p < 0.001$ ), total N ( $r = 0.41$ ,  $p < 0.05$ ), net total N mineralized ( $r = 0.35$ ,  $p < 0.05$ ) and total P ( $r = 0.67$ ,  $p < 0.001$ ).  $\text{NH}_4^+\text{-N}$  positively correlated with  $\text{NO}_3^-\text{-N}$  ( $r = 0.86$ ,  $p < 0.001$ ), total N ( $r = 0.41$ ,  $p < 0.05$ ), net total N mineralized ( $r = 0.99$ ,  $p < 0.001$ ) and total P ( $r = 0.31$ ,  $p < 0.05$ ), while  $\text{NO}_3^-\text{-N}$  correlated positively with total N ( $r = 0.44$ ,  $p < 0.05$ ), net total N mineralized ( $r = 0.88$ ,  $p < 0.001$ ) and total P ( $r = 0.47$ ,  $p < 0.05$ ), and net total N mineralized positively correlated with total P ( $r = 0.33$ ,  $p < 0.05$ ). Total N positively correlated with net total N mineralized ( $r = 0.66$ ,  $p < 0.05$ ) and total P ( $r = 0.47$ ,  $p < 0.05$ ). Lastly, a positive significant correlation was observed between net extractable P mineralized and total P ( $r = 0.57$ ,  $p < 0.05$ ).

On the other hand, significant negative correlations were observed between total C and C: P ( $r = -0.43$ ,  $p < 0.05$ ), lignin ( $r = -0.28$ ,  $p < 0.05$ ), lignin: N ( $r = -0.35$ ,  $p < 0.05$ ). C: N correlated negatively with  $\text{NH}_4^+\text{-N}$  ( $r = -0.34$ ,  $p < 0.05$ ),  $\text{NO}_3^-\text{-N}$  ( $r = -0.35$ ,  $p < 0.05$ ), total N ( $r = -0.91$ ,  $p < 0.001$ ), net total N mineralized ( $r = -0.75$ ,  $p < 0.05$ ) and total P ( $r = -0.26$ ,  $p < 0.05$ ). C: P negatively correlated with  $\text{CO}_2\text{-C}$  ( $r = -0.49$ ,  $p < 0.05$ ),  $\text{NO}_3^-\text{-N}$  ( $r = -0.24$ ,  $p < 0.05$ ), total N ( $r = -0.43$ ,  $p < 0.05$ ), net extractable P mineralized ( $r = -0.73$ ,  $p < 0.05$ ) and total P ( $r = -0.92$ ,  $p < 0.001$ ). Lignin negatively correlated with  $\text{CO}_2\text{-C}$  ( $r = -0.60$ ,  $p < 0.001$ ), total N ( $r = -0.43$ ,  $p < 0.05$ ) and total P ( $r = -0.29$ ,  $p < 0.05$ ) while lignin: N correlated negatively with  $\text{CO}_2\text{-C}$  ( $r = -0.55$ ,  $p < 0.001$ ),  $\text{NH}_4^+\text{-N}$  ( $r = -0.24$ ,  $p < 0.05$ ), lignin: N ( $r = -0.89$ ,  $p < 0.001$ ) and total P ( $r = -0.51$ ,  $p < 0.001$ ) (Table 5.5).

**Table 5. 5:** Pearson's rank correlation between selected biochemical properties of wheat residues and incubation variables

	TC%	C: N	C: P	Lignin %	Lignin: N	CO <sub>2</sub> -C(mg kg <sup>-1</sup> soil)	NH <sub>4</sub> <sup>+</sup> -N(mg kg <sup>-1</sup> soil)	NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> soil)	TN%	N Min(mg kg <sup>-1</sup> soil)	P Min(mg kg <sup>-1</sup> soil)	TP %
<b>C_%</b>	1.00											
<b>C_N</b>	0.153	1.00										
<b>C_P</b>	-0.43*	0.26*	1.00									
<b>Lignin%</b>	-0.28*	0.37*	0.15*	1.00								
<b>Lignin:N</b>	-0.35*	0.78**	0.43*	0.74**	1.00							
<b>CO<sub>2</sub>-C(mg kg<sup>-1</sup>soil)</b>	0.61*	-0.23	-0.49*	-0.60**	-0.55**	1.00						
<b>NH<sub>4</sub><sup>+</sup>-N(mg kg<sup>-1</sup>soil)</b>	0.27*	-0.34*	-0.23	-0.03	-0.24*	0.35*	1.00					
<b>NO<sub>3</sub><sup>-</sup>-N(mg kg<sup>-1</sup>soil)</b>	0.11	-0.35*	-0.24*	-0.03	-0.24	0.51**	0.86**	1.00				
<b>N_%</b>	0.21	-0.91**	-0.43*	-0.43*	-0.89**	0.41*	0.45*	0.44*	1.00			
<b>N Min(mg kg<sup>-1</sup>soil)</b>	0.25*	-0.75*	-0.26*	0.01	-0.23	0.35*	0.99**	0.88**	0.66*	1.00		
<b>P Min(mg kg<sup>-1</sup>soil)</b>	0.16	-0.08	-0.73*	0.02	-0.16	0.05	-0.02	-0.07	0.22	-0.02	1.00	
<b>TP%</b>	0.52**	-0.26*	-0.92**	-0.29*	-0.51**	0.67**	0.31*	0.32*	0.47*	0.33*	0.57*	1.00

Significant at p<0.05= \*, Significant a p<0.001=\*\*:% TN=total nitrogen in residue tissues,% TC=total nitrogen in residue tissues,C:N=C:N ratio of wheat residues,C: P= C: P ratio of wheat residues,CO<sub>2</sub>-C=Net CO<sub>2</sub>-C evolved by residue, Lignin%=Lignin content of residues,Lignin:N=Lignin:N ratio of wheat residues,NH<sub>4</sub><sup>+</sup>-N= Net NH<sub>4</sub><sup>+</sup>-N concentration released by residues and NO<sub>3</sub><sup>-</sup>-N= Net NO<sub>3</sub><sup>-</sup>-N concentration released by the residues, N min= net total N mineralized by residues and P min= Net P mineralized by residues

## 5.5 Discussion

### *5.5.1 Variation of CO<sub>2</sub>-C emission amongst different wheat residues*

Net CO<sub>2</sub>-C emission rates were higher in the first two weeks, but declined after day 21, reaching smaller peaks (Figure 5.1). The rapid increase of CO<sub>2</sub>-C in the first two weeks of incubation could be due to the opportunistic and colonizer organisms which grow and reproduce rapidly in environments with plant material. The decrease in residue mineralization in later stages may indicate that more C was sequestered in the soil or was incorporated into microbial biomass. These results are consistent with previous work by Potthoff et al., (2005), who reported a decline in CO<sub>2</sub>-C emission with increasing incubation period in incubated maize straw. Also, similar results were found by Henriksen and Breland (1999) in their incubation study of wheat straw. In this study, incorporation of wheat residues increased net CO<sub>2</sub>-C evolution compared to soil alone (control). These results also corroborate the results obtained by Potthoff et al. (2005), who found out that mixing of the maize straw with soil caused almost 40% increase in CO<sub>2</sub>-C production than in controls.

Net CO<sub>2</sub>-C emissions stabilized after day 77 of incubation simply because the majority of labile C had been consumed by microbial biomass (de Almeida et al., 2014). Oscillations or small peaks in CO<sub>2</sub>-C emission after day 21 could be due to the succession and stability of microbial organism communities (Moreira and Siqueira, 2006). The shoots of BW140 and BW162 released higher amounts of net CO<sub>2</sub>-C compared with other treatments. This corresponds with the high total tissue nitrogen and low C: N or lignin: N ratios (Table 5.2) obtained in these residues compared with other treatments, which favoured rapid microbial decomposition. Moreover, these residues could also contain high amounts of soluble or labile carbon. Tanvea and Gozalez-Meler (2008) reported that organic matter added to soil containing high amounts of labile carbon potentially enhances CO<sub>2</sub> emission and thus restricts accumulation of carbon

in the soil. The higher net CO<sub>2</sub>-C emission of BW140 ST and BW162 ST indicated that they provided more easily degradable C and potentially more soluble C for microbial activity than other wheat residues.

The trend of net CO<sub>2</sub>-C emission from these treatments declined after 21 days of incubation, however in general it might take a longer time for microbial activity to start after incorporation of residues under real field conditions (Reis et al., 2011). The study was conducted under laboratory conditions therefore data might not correlate with field conditions because of environmental heterogeneity. The decline of net CO<sub>2</sub>-C emission with time was also reported by Dong et al. (2009), who suggested that lower CO<sub>2</sub> emission was due to slower decomposition process in the presence of recalcitrant compounds such as lignin as incubation progressed.

In this study net CO<sub>2</sub>-C emission was generally lower in treatments containing roots (Figure 5.1), with LM70 RT releasing the lowest amounts of net CO<sub>2</sub>-C (48.80 mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) (Table 5.3). This could be attributed to the biochemical composition of these residues, as they exhibited significantly higher lignin content in the roots compared to shoots, while BW162 ST and BW140 ST treatments with the least Lignin: N as well as C: N ratios showed more decomposition potential with higher net CO<sub>2</sub>-C emissions. Walli et al., (1988) reported that biochemical composition of organic residues affects their microbial degradation. Thus, higher lignin content in roots slowed down microbial degradation thereby limiting CO<sub>2</sub> emission. Lignin content and lignin: N ratio negatively correlated with Net CO<sub>2</sub>-C ( $r = -0.60$ ,  $p < 0.001$ ) and ( $r = -0.55$ ,  $p < 0.001$ ) (Table 5.5), these results suggested that net CO<sub>2</sub>-C evolution decrease with increasing lignin content and lignin: N ratio. Therefore, this relationship confirms the



lower CO<sub>2</sub>-C evolution of root treatments as they exhibited higher lignin concentration and higher lignin: N ratio than shoot treatments (Table 5.2).

Net cumulative CO<sub>2</sub>-C emission was also higher in the shoots than in roots (Figure 5.2), and this was closely related to lower lignin: N as well as C: N ratios of shoot residue treatments. Similar results were obtained by Shaaban et al., (2016), who reported high CO<sub>2</sub>-C emission from above ground biomass compared to the below ground biomass.

### ***5.5.2 Variation on N mineralization amongst different wheat residues***

Net ammonium (NH<sub>4</sub><sup>+</sup>-N) concentration increased rapidly and reached a maximum at day 55 of incubation and gradually decreased afterwards while net nitrate (NO<sub>3</sub><sup>-</sup>-N) continuously increased during the entire incubation period (Figure 5.3 & 5.4). This rapid increase of net NH<sub>4</sub><sup>+</sup>-N concentration is attributable to the decomposition of easily decomposable nitrogenous substances (amino sugars, nucleic acids and proteins) present in organic material. These results were in corroboration with the findings of Nagaraja (1988). The gradual decrease of net NH<sub>4</sub><sup>+</sup>-N concentration after 55 days of incubation could be brought about by the process of nitrification as it had been reported to cause a decrease in the concentration of NH<sub>4</sub><sup>+</sup>-N, similarly the increase of net NO<sub>3</sub><sup>-</sup>-N concentration throughout the incubation period could be attributed to the activity of nitrifying bacteria which convert NH<sub>4</sub><sup>+</sup>-N to NO<sub>3</sub><sup>-</sup>-N (Murugan and Swarnam, 2013).

It was observed from the study that the amount of net total N mineralized (NH<sub>4</sub><sup>+</sup>-N +NO<sub>3</sub><sup>-</sup>-N) differed amongst the residues of wheat genotypes (Figure 5.5 & Appendix AH.1). This could be due to the variation of total tissue N resulting in variation of C: N ratios in plant parts of wheat genotypes. As expected, the plant parts also affected net total N mineralization, with roots displaying a significantly lower net total mineralized N compared to shoots (Figure 5.5).

Incorporation of BW162 ST, BW140 ST, BW152 ST and BW140 RT resulted in the highest, while the application of BW152 RT resulted in the lowest net total mineralized N (Table 5.4, Figure 5.5). This could be attributed to the difference of their C: N ratios. BW162 ST, BW140 ST, BW152 ST and BW140 RT exhibited higher concentration of total tissue N and lower C: N ratios (Table 5.2). Low C: N ratio causes rapid mineralization of organic N, whereas high C: N in residues results in the immobilization of mineral N as it is utilized by microbial biomass (Schimel et al., 1992; Mary et al., 1996). A high C: N ratio of > 40 is known to increase the potential for N immobilization in the soil (Baldock, 2007). In this present study BW152 RT had a very high C: N ratio of 89.44 suggesting a high probability of N immobilization. Similarly, Das et al. (1993) found that sorghum stover (C: N=72) resulted in immobilization of N for 90 days of incubation.

Net total N mineralized positively correlated with total N ( $r= 0.66$ ,  $p<0.05$ ) and negatively correlated with C: N ratio ( $r= -0.75$ ,  $p<0.05$ ) of residues (Table 5.5). These results suggest that N mineralization increases with increasing N content in residues and decreases with increasing C: N ratio. Similar findings have been reported by several studies (Kumar and Goh, 2003; Soon and Arshad, 2002; Lupwayi and Haque 1999).

### ***5.5.3 Variation of P mineralization amongst different wheat residues***

In this study net P mineralization increased with increasing days of incubation until a stability was reached (Figure 5.6). This was due to that most incubated residues except for BW140 root exhibited total initial P content greater than 0.2% (Table 5.2) which indicates potential mineralization. According to Floate (1970), residues with P values <0.2% show low or no net P mineralization which resulted in BW140 roots residues mineralizing the least net mineral P. In a study by Mafongoya et al. (2000), net P immobilization was observed when leaves of

agroforestry tree species (*Gliricidia sepium*, *Acacia nilotica*) containing total P of <0.2% were incubated with soil. Net P mineralized positively correlated with total P content in residues ( $r=0.57$ ,  $p<0.05$ ), while it negatively correlated with C: P ( $r=-0.73$ ,  $p<0.05$ ) (Table 5.5). These results suggested that P mineralization increases with increasing P content of the residues and decreases with increasing C: P ratio. In the present study net P mineralized was higher in shoot treated soils compared to root treated soils (Table 5.3). That could be attributed to the higher initial content of total P present in the shoot tissues than in root tissues of the studied wheat residues. Net P mineralized was highest in BW152 shoots ( $3.69 \text{ mg P kg}^{-1} \text{ soil}$ ) and lowest in BW140 roots ( $0.82 \text{ mg P kg}^{-1} \text{ soil}$ ). That could be due to that BW152 shoots had higher initial total P content and lower C: P ratio while BW140 roots exhibited lower initial total P content and highest C: P (Table 5.2), which resulted in BW152 shoots mineralizing high P and BW140 roots mineralizing lowest P (Table 5.4 & Figure 5.6). Damon et al. (2014) also demonstrated that P mineralisation is increased for residues with high initial P content.

## 5.6 Conclusion

The incorporation of residues of different wheat genotypes had significant effects on CO<sub>2</sub> emission, N and P mineralization. It resulted in higher CO<sub>2</sub> emissions than in soil alone. LM70 roots evolved the lowest while BW140 and BW162 shoots evolved the highest net CO<sub>2</sub>-C. Therefore, LM70 root residues are advisable to farmers for sequestering C into the soil. On the other hand, incorporation of BW162, BW140 and BW152 shoots resulted in higher net total N mineralization whilst BW152 roots gave the lowest net total N mineralization. BW162 ST, BW152 ST and LM75 ST also gave higher net mineralized P while BW140 RT had the lowest net mineralized P. It was recommended that BW162, BW140 and BW152 shoots could be a good source of N while BW162, BW152 and LM75 shoots are ready sources of mineral P into the soil and could serve as potential organic fertilizers.

## **CHAPTER SIX**

### **GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS**

#### **6.1 Chapter outline**

This chapter gives a synthesis of major findings as well as conclusions drawn, and recommendations made from the study. It begins by giving an overview of the environmental factors affecting WUE of different crops at a global scale from a meta-analysis. It then documents the effects of different scenarios of water availability on WUE of different wheat genotypes and lastly look at the biochemical factors affecting residue decomposition and nutrient release patterns of wheat from different genotypes.

#### **6.2 General discussion**

Climate change predictions indicate reduced, highly erratic and unevenly distributed rainfall patterns coupled with increasing temperatures in South Africa (Schulze, 2011). The reduced, erratic and unevenly distributed rainfall patterns have resulted in limited water availability thus reduced crop production. In addition to limited water availability, poor soil fertility has also been identified as another constraint to crop production in the SSA (Sanchez et al., 1997). Therefore, this implies development of strategies for adaptation to this unfavourable climate and improving soil fertility to enhance crop production and food security. Such strategies include improving crop water use efficiency (WUE) and the usage of crop residues to restore soil carbon and recycling of soil nutrients. The aim of this study was to assess water use efficiency and carbon sequestration potential of different wheat genotypes.

Several studies have reported on crop WUE variations with crop types, agricultural practices, soil type, climate, amount of irrigation water applied and growing season. However, the data

from such studies across the world provided an opportunity for comprehensive analysis to draw a general understanding of factors affecting crop WUE at a global scale.

Findings from the meta-analysis of global data indicated that maize and sorghum had higher WUE than other cereals such as wheat. These differences could be explained by their C4 photosynthetic pathway which is different from that of wheat, a C3 plant. Plants exhibiting a C4 pathway have been found to have higher water use efficiency than C3 plants across many studies (Blankenagel et al., 2018; Way et al., 2014). This higher WUE is attributable to the increased CO<sub>2</sub> concentration mechanism in the special bundle sheath cells of C4 crops, which allows higher photosynthetic rates while reducing stomatal conductance and transpiration resulting in water savings (Sage and Monson, 1999). The differences in photosynthetic pathway in crops further resulted in different WUE for summer and winter grain crops. This was because summer grain crops were dominated by C4 crops (maize, sorghum and millet), while winter grains were dominated by C3 crops (wheat and barley). The meta-analysis findings also showed that grain crops exhibited higher WUE than legumes, oilseed and fibre crops which was anticipated as grain crops were dominated by C4 crops. Similar findings were also reported by Angadi et al. (2008).

The meta-data analysis also showed that climate had a significant impact on WUE of different crops. Thus, crop WUE tended to increase from desert to subtropical to tropical environments. These climates (desert, subtropical and tropical) are characterized by warm temperatures, therefore the increased crop WUE was mainly due to high temperatures prevailing in these environments. Warm temperatures have been reported to increase crop WUE as they increase photosynthetic rate (Zhang et al., 2015). The amount of irrigation water applied also significantly affected crop WUE, thus it was higher under both low and high-water application. This was attributed to different physiology amongst crop types, as some crops would thrive

and adapt easily under water stressed conditions while others would thrive under well-watered conditions. Some studies have reported increase in WUE under stressed conditions (Chibarabada et al., 2015) while others have reported higher WUE under non-stressed conditions (Boutraa et al., 2010). High water use efficiency of bambara groundnut under stressed conditions in a study conducted by Chibarabada et al. (2015) was attributed to drought tolerance attributes (i.e. reduced leaf number, leaf surface area, plant height and sugar accumulation) observed in stressed bambara seedlings. While high WUE of some wheat cultivars under non-stressed conditions in a study by Boutraa et al. (2010) was attributed to the drought sensitivity of these wheat cultivars, which caused their WUE to be low under water stressed conditions and higher under non-stressed conditions. Therefore, the impact of watering regime on crop WUE depends on the crop type and its adaptability and sensitivity to that volume of water applied.

The meta-analysis findings also showed that water use efficiency (WUE) of different crops is affected by soil texture. It was higher in silt loam and clay loam soils compared to clayey textured soils. This was mainly due to that high clay content in soils may create waterlogged conditions which reduce gaseous exchange. This then hinders plant growth as well as transport of nutrients and water to upper plant parts, causing low yields and death of crops in some instances. Katerjie et al. (2009) also reported higher crop WUE under loam textured soil compared to clay soil. However, this also depends on the crop type and its adaptation to a particular soil type. Hence, some crops would grow well and exhibit high WUE when grown on clayey textured soils. In our meta-analysis, maize exhibited higher WUE than all the other crops when grown in clayey soil.

Though WUE has been reported to be lower under crops with a C3 photosynthetic pathway than C4 crops, there are some C3 crops such as wheat which are staple food to many and are

important in South Africa's economy, therefore attempts must be made to enhance their productivity. There is evidence to show that WUE also varies among different crop cultivars of the same crop (Mudenda et al. 2016; Boutraa et al. 2010; Zhang et al. 2010). Thus, other than promoting C4 crop types with superior WUE only, efforts must also be made towards breeding more water use efficient cultivars of C3 crops. Climate change predictions also indicate that carbon dioxide in the atmosphere would be doubled by 2050 if the current rate of CO<sub>2</sub> increase continues, causing the world temperature to rise by 2-4°C (IPCC 2013). However, the direct effect of CO<sub>2</sub> on plants is positive (Warrick, 1988), as a result they could be a potential sink for CO<sub>2</sub>. Crops exhibiting C3 pathway have been reported to generally have lower WUE than C4 crops, but their response to enhanced CO<sub>2</sub> is positive (Leakey, 2009). Increased CO<sub>2</sub> in the atmosphere decreases transpiration of C3 plants as they partly close their stomata thereby reducing plant water losses (Kimball et al., 2002). Wheat is a C3 plant and is the second most important grain crop after maize in South Africa (DAFF 2010). However, its production is hampered by an inherently dry climate as annual rainfall for South is generally low, averaging 495 mm (FAO, 2005), making water availability a limiting factor to crop production. There is therefore a need to select and grow wheat genotypes which are drought tolerant, with high abilities to sequester CO<sub>2</sub> from the atmosphere, thereby improving soil carbon stocks through decaying plant litter as well as root exudates. One of the major limitations of our meta-analysis was that it only found studies on water use efficiency for grain yield (GY) and total plant biomass (PB) production, but not that of plant carbon stock (PCs) which is also crucial. It was thus essential to complement the meta-analysis findings with more detailed field, glasshouse and laboratory incubation studies that compared wheat genotypes for WUE with reference to plant carbon stocks and their potential to sequester soil C.

Our findings showed that, water use efficiency for grain yield production (WUE-GY), total plant biomass production (WUE-PB) and total plant carbon stocks production (WUE-PCs)



varied within the wheat genotypes and were affected by different water regimes. Both WUE-GY and WUE-PB were higher under stressed conditions. Similar findings were obtained by Chibarabada et al., (2015) and Abbate et al., (2004). This could be attributed to reduced stomatal aperture and transpiration rate of wheat under stressed conditions and also to plant roots extracting more soil water from greater depth under conditions of moisture stress compared to when they are irrigated (Pirasteh-Anosheh et al., 2016; El Hwary and Yagoub, 2011). Amongst the wheat genotypes studied, LM75 exhibited highest WUE-GY under stressed conditions, whilst BW141 exhibited lower WUE-GY. Under non-stressed conditions genotype BW162 exhibited highest WUE-GY whilst LM48 and LM47 exhibited lower WUE-GY. As for WUE-PB, genotype LM75 exhibited highest WUE-PB under stressed conditions, whilst LM71 and LM48 exhibited the lowest WUE-PB. Under non-stressed conditions BW140 had higher WUE-PB while LM47 exhibited the lowest WUE-PB. These findings revealed that the genotype LM75 was not sensitive to water stress for production of grain and total plant biomass whilst LM71, LM48 and LM47 were sensitive and are therefore not the best genotypes for grain yield and total plant biomass production when water supply is limited. Drought tolerant wheat cultivars are reported to show an increase in WUE under water stress conditions, while drought sensitive cultivars will show decreased WUE (Michirio et al., 1994). Water use efficiency for total plant carbon stocks (WUE-PC<sub>s</sub>) for most genotypes was also higher under water stressed compared to non-stressed conditions. This could be due to the fact that under stressed conditions, there is decrease in stomatal conductance and an increase in photosynthetic rate, therefore more carbon is assimilated, while less water is transpired (Farquhar et al., 1980; Zhang et al., 2012). Under stressed conditions WUE-PC<sub>s</sub> was highest in genotypes BW162 and LM75 and lowest in LM71, while under non-stressed conditions BW140 exhibited the highest WUE-PC<sub>s</sub> and LM71 had the lowest. Therefore, from these findings it was deduced that

drought tolerant wheat cultivars assimilate more carbon with less water resulting in higher carbon storage in their tissues.

The disparities of plant carbon stocks amongst different wheat genotypes suggested that residues from these genotypes exhibit different rates of decomposition and nutrient release patterns. However, not much was known about the decomposition potential and nutrient release patterns of these wheat genotypes once incorporated into the soil. Crop residues have been identified as one of the common organic sources that are readily available to most farmers (Hossener and Juo, 1999). Incorporation of crop residues into the soil directly contributes to soil organic matter (SOM) build-up which is crucial for improving crop yields and cycling of nutrients into the soil system (Soon and Arshad, 2002; Boem and Anderson, 1997). Therefore, as poor soil fertility is also a constraint to crop production in some parts of SSA, thus crop residues could be a readily available source of nutrients to farmers. According to Sinha et al. (2018), about 25% of N and P, 50% of S as well as 75% of K taken up by cereal crops are stored in residues, which makes them viable sources of nutrients in the soil. Wheat residues could therefore be incorporated into the soil after harvest to release these nutrients retained in its tissues during decomposition. This could reduce environmental damage caused by continued use of chemical fertilizers as they have been reported to cause SOM deterioration, soil acidification and death of beneficial soil microorganisms (Gruhn et al., 2000; Setboonsang, 2006).

Though crop residues are a valuable source of nutrients, knowing their biochemical composition before incorporating them back into soil is important, since this controls their decomposition process. The carbon (C), nitrogen (N), lignin, cellulose, polyphenol contents and C: N ratio are good indicators of plant residue quality and its ease of decomposition. According to Baldock (2007), plant residues with a high C: N ratio (>40) are mineralized far

more slowly than residues with a C: N ratio of less than 40. High lignin content in residues also results in slower decomposition rate (Berg et al., 2010), while aboveground biomass (e.g. leaves and stems) give high quality residues compared to below ground biomass (e.g. roots) which are relatively recalcitrant to decomposition (Bertrand et al., 2006). The root residues are decomposed slowly and therefore contribute largely to SOM (Johnson et al., 2014). The variation of biochemical quality between shoot and root residues from different wheat genotypes was observed and resulted in their different decomposition patterns after incorporation into soil. Findings showed that lignin content and the C: N ratio was higher in root compared to shoot residues for most wheat genotypes. This resulted in root residues releasing lower net CO<sub>2</sub>-C for all wheat genotypes, while also mineralizing lower net N and extractable P for most genotypes.

The shoot residues of genotypes BW140 and BW162 released higher amounts of net CO<sub>2</sub>-C compared with other treatments. This corresponded with the high total N content, lower C: N ratios and lower lignin content of these shoots which favoured rapid microbial decomposition thus releasing high quantities of net CO<sub>2</sub>-C. Again, genotype BW140 was ranked the highest for production of shoot biomass (SB), total plant biomass (PB), shoot carbon stocks (SC<sub>s</sub>) and total plant carbon stocks (PC<sub>s</sub>) (in chapter 3), which could also be the reason for its high CO<sub>2</sub> release. Root residues of genotype LM70 released the lowest net CO<sub>2</sub>-C. This was attributed to significantly higher lignin content present in the roots of this genotype. Higher lignin in plant tissue is known to slow down microbial degradation, limiting CO<sub>2</sub> emission. The slow decomposition of LM70 roots indicated that more soil carbon was sequestered by these residues. Incorporation of shoot residues from genotypes BW162, BW140 and BW152 as well as root residues of BW140 retained highest net mineral N into the soil. This was because of their higher initial concentration of total N and lower C: N ratio in their tissues. Low C:N ratio causes ready mineralization of organic N, whereas high C:N ratio of residues results in the

immobilization of mineral N as it is utilized by microbial biomass (Schimel et al., 1992; Mary et al., 1996). Incorporation of root residues from genotype BW152 retained the lowest net mineral N into the soil, exhibiting a very high probability of N immobilization in soil due to its high C: N ratio. Net P mineralization of wheat residues was highest in shoots of BW152, LM75 and BW162 but lowest in roots of LM75, BW152 and BW140. This was related to the initial P content and C: P ratio of these residues. Thus, residues with high P content and low C: P ratio mineralized highest P while residues with low P content and high C: P ratio mineralized the lowest P amounts.

### **6.3 Conclusion and recommendations**

Water use efficiency varies for different crop types, with crops exhibiting a C4 photosynthetic pathway having higher WUE than C3 plants. The prevailing climate in an area also has an impact on crop WUE. Thus, crops grown in environments of higher temperatures exhibited higher WUE than crops grown in cool environments. Field and glasshouse studies also confirmed that WUE of wheat genotypes differed in response to water availability. The incorporation of root and shoot residues from different wheat genotypes also proved to have a significant effect on net CO<sub>2</sub>-C evolution and mineralization of nutrients. Findings showed that roots produced less biomass and evolved lower CO<sub>2</sub>-C than shoots, and hence have potential for more soil C sequestration.

The study recommends that in warm environments (tropical, subtropical and deserts) growth of crops exhibiting a C4 photosynthetic pathway (i.e. maize, sorghum) should be promoted since they tend to exhibit higher WUE under water stressed and hot conditions. Also, when

considering wheat production in regions with limited water availability, the genotype LM75 is an ideal candidate for grain and total biomass production of wheat since it is more tolerant to drought stress. On the other hand, the wheat genotypes BW162 and LM75 are ideal to build plant carbon stocks since they had high WUE-PCs under water-stressed conditions. In addition, the root residues of LM70 are advisable to farmers for sequestering C into the soil and for building up soil organic matter reserves. Shoot residues from genotypes BW162, BW140 and BW152 can also be used as a source of N in the soil since they released higher amounts of mineral N than other residues. While shoot residues from BW162, BW152 and LM75 also mineralized higher P compared to other genotypes, therefore they can be good sources of P into the soil.

Future research should focus on selecting for the most water use efficient maize and sorghum genotypes since they are also important cereals, commonly produced by smallholder farmers residing in arid to semi-arid areas of South Africa, that often face challenges of limited water availability. A meta-analysis focusing on the environmental factors controlling WUE of crops commonly grown in South African could also be conducted. This will aid in knowing which crops are more water use efficient in the South African context. While this work showed potential for using wheat residues as a SOM remedy and a source of N and P, its impact will depend on the volumes of selected wheat genotypes residues available in the local environments. Therefore, research on the available quantities of wheat residues from the selected genotypes in the local environments of South Africa is required.

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## APPENDICES

### Appendix A. 1: The ANOVA GY for 100 genotypes in the field as influenced by water availability (25%FC) and (75%FC)

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	417650.	4219.	2.36	
REP.*Units* stratum					
Water regime	1	47444182.	47444182.	171.80	<.001
GENOTYPE	99	59030045.	596263.	2.16	<.001
Water reg.GENOTYPE	99	31070907.	313848.	1.14	0.224
Residual	199	54955297.	276157.		

### Appendix B. 1: The ANOVA of PB of 100 different wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)

Variate: PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	278938.	2818	1.82	
REP.*Units* stratum					
Water regime	1	372807612.	372807612.	323.86	<.001
ENTRY	99	246422361.	2489115.	2.16	<.001
Water reg.GENOTYPE	99	118426023.	1196222.	1.04	0.405
Residual	199	229076646.	1151139.		

**Appendix C. 1: The ANOVA of R: S of 100 different wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: R:S

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.008849.	0.000089	0.14	
REP.*Units* stratum					
Water regime	1	0.004113	0.004113	2.31	0.130
GENOTYPE	99	0.455857	0.004605	2.59	<.001
Water reg.GENOTYPE	99	0.249765	0.002523	1.42	0.020
Residual	199	0.353873	0.001778		

**Appendix D. 1: The ANOVA of GY for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	150706.	150706.	1.44	
REP.*Units* stratum					
Water regime	1	15219522.	15219522.	145.06	<.001
GENOTYPE	9	6905840.	767316.	7.31	<.001
Water reg.GENOTYPE	9	4473780.	497087.	4.74	0.002
Residual	19	1993447.	104918.		

**Appendix E. 1: The ANOVA of PB for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	191446.	191446.	1.66	
REP.*Units* stratum					
Water regime	1	69826621.	69826621.	604.86	<.001

GENOTYPE	9	34952121.	3883569.	33.64	<.001
Water reg.GENOTYPE	9	14410664.	1601185.	13.87	<.001
Residual	19	2193395.	115442.		

**Appendix F. 1: The ANOVA of GCC for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: GCC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	2.7584	2.7584	9.79	
REP.*Units* stratum					
Water regime	1	0.0000	0.0000	0.00	1.000
GENOTYPE	9	105.2663	11.6963	41.53	<.001
Water reg.GENOTYPE	9	0.0000	0.0000	0.00	1.000
Residual	19	5.3514	0.2817		

**Appendix G. 1: The ANOVA of RCC for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: RCC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	104.575	104.575	24.19	
REP.*Units* stratum					
Water regime	1	0.015	0.015	0.00	0.954
GENOTYPE	9	571.014	63.446	14.68	<.001
Water reg.GENOTYPE	9	0.136	0.015	0.00	1.000
Residual	19	82.122	4.322		

**Appendix H. 1: The ANOVA of SCC for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: SCC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	1.287	1.287	0.31	

REP.*Units* stratum					
Water regime	1	2.016	2.016	0.48	0.497
GENOTYPE	9	123.053	13.673	3.25	0.015
Water reg.GENOTYPE	9	18.126	2.014	0.48	0.871
Residual	19	79.982	4.210		

**Appendix I. 1: The ANOVA of GY for the selected 10 wheat genotypes grown in the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2436.	1218.	0.11	
REP.*Units* stratum					
Water regime	1	116794.	116794.	10.88	0.002
GENOTYPE	9	961228.	106803.	9.94	<.001
Water reg.GENOTYPE	9	1122858.	124762.	11.62	<.001
Residual	38	408100.	10739.		

**Appendix J. 1: The ANOVA of PB for the selected 10 wheat genotypes grown in the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	215888.	107944.	2.88	
REP.*Units* stratum					
Water regime	1	163105548.	163105548.	4355.44	<.001
GENOTYPE	9	43200326.	4800036.	128.18	<.001
Water reg.GENOTYPE	9	33464985.	3718332.	99.29	<.001
Residual	38	1423051.	37449.		

**Appendix K. 1: The ANOVA of WUE for GY for the selected 10 wheat genotypes grown in the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.002316	0.001158	0.36	
REP.*Units* stratum					
Water regime	1	0.174858	0.174858	53.99	<.001
GENOTYPE	9	0.337658	0.037518	11.58	<.001
Water reg.GENOTYPE	9	0.349187	0.038799	11.98	<.001
Residual	38	0.123076	0.003239		

**Appendix L. 1: The ANOVA of WUE for GY for the selected 10 wheat genotypes grown in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.4051	0.4051	1.15	
REP.*Units* stratum					
Water regime	1	4.9139	4.9139	13.94	0.001
GENOTYPE	9	1.2042	0.1338	0.38	0.931
Water reg.GENOTYPE	9	2.1598	0.2400	0.68	0.717
Residual	19	6.6961	0.3524		



**Appendix M. 1: The ANOVA of WUE for PB for the selected 10 wheat genotypes grown in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.387	0.387	0.31	
REP.*Units* stratum					
Water regime	1	28.025	28.025	22.76	<.001
GENOTYPE	9	5.397	0.600	0.49	0.865
Water regim.GENOTYPE	9	6.785	0.754	0.61	0.772
Residual	19	23.390	1.231		

**Appendix N. 1: The ANOVA of WUE for PC<sub>s</sub> for the selected 10 wheat genotypes grown in the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_PC<sub>s</sub>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.11016	0.05508	4.47	
REP.*Units* stratum					
Water regime	1	0.89720	0.89720	72.89	<.001
GENOTYPE	9	2.82561	0.31396	25.51	<.001
Water reg.GENOTYPE	9	2.39779	0.26642	21.65	<.001
Residual	38	0.46772	0.01231		

**Appendix O. 1: The ANOVA of Grain yield of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	59521670.	14880417.	34.39	
REP.*Units* stratum					
Water regime	1	4492776.	4492776.	10.38	0.002
GENOTYPE	9	16746533.	1860726.	4.30	<.001
Water reg.GENOTYPE	9	2717401.	301933.	0.70	0.709
Residual	76	32886903.	432722.		

**Appendix P. 1: The ANOVA of root biomass different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: RB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	30024096.	7506024.	33.32	
REP.*Units* stratum					
Water regime	1	18242912.	18242912.	80.99	<.001
GENOTYPE	9	5469565.	607729.	2.70	0.009
Water Reg.GENOTYPE	9	4689496.	521055.	2.31	0.023
Residual	76	17119433.	225256.		

**Appendix Q. 1: The ANOVA of shoot biomass of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: SB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	8475945.	2118986.	2.62	
REP.*Units* stratum					
Water regime	1	61207105.	61207105.	75.58	<.001
GENOTYPE	9	36965189.	4107243.	5.07	<.001
Water reg.GENOTYPE	9	8126037.	902893.	1.11	0.363
Residual	76	61547077.	809830		

**Appendix R. 1: The ANOVA of total plant biomass of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	13224084.	3306021.	1.31	
REP.*Units* stratum					
Water regime	1	163949744.	163949744.	64.75	<.001
GENOTYPE	9	40902367.	4544707.	1.79	0.083
Water reg.GENOTYPE	9	29165421.	3240602.	1.28	0.262
Residual	76	192432907.	2532012.		

**Appendix S. 1: The ANOVA of WUE for GY of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_GY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	5.9574	1.4894	9.11	
REP.*Units* stratum					
Water regime	1	5.5924	5.5924	34.20	<.001
GENOTYPE	9	2.1417	0.2380	1.46	0.180
Water reg.GENOTYPE	9	1.3403	0.1489	0.91	0.521
Residual	76	12.4287	0.1635		

**Appendix T. 1: The ANOVA of WUE for RB of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_RB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	40.27587	10.06897	115.93	
REP.*Units* stratum					
Water regime	1	0.08589	0.08589	0.99	0.323
GENOTYPE	9	4.38118	0.48680	5.61	<.001
Water reg.GENOTYPE	9	3.21047	0.35672	4.11	<.001
Residual	76	6.60064	0.08685		

**Appendix U. 1: The ANOVA of WUE for SB of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_SB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	5.79331	1.44833	57.39	
REP.*Units* stratum					
Water regime	1	1.34633	1.34633	53.35	<.001
GENOTYPE	9	1.38903	0.15434	6.12	<.001
Water reg. GENOTYPE	9	0.33197	0.03689	1.46	0.178
Residual	76	1.91789	0.02524		

**Appendix V. 1: The ANOVA of WUE for PB of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_PB

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	119.7372	29.9343	30.21	
REP.*Units* stratum					
Water regime	1	32.6424	32.6424	32.94	<.001
GENOTYPE	9	25.3045	2.8116	2.84	0.006
Water regime.GENOTYPE	9	12.5224	1.3914	1.40	0.201
Residual	76	75.3112	0.9909		

**Appendix W. 1: The ANOVA of WUE for GCs of different wheat genotypes across the field and the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_GC<sub>s</sub>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	0.84766	0.21192	20.44	
REP.*Units* stratum					
Water regime	1	0.54066	0.54066	52.15	<.001
GENOTYPE	9	1.15655	0.12851	12.39	<.001
Water reg.GENOTYPE	9	0.25620	0.02847	2.75	0.008
Residual	76	0.78795	0.01037		

**Appendix X. 1: The ANOVA of WUE for RCs of different wheat genotypes across the field and glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_RC<sub>s</sub>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	3.678594	0.919649	131.72	
REP.*Units* stratum					
Water regime	1	0.006359	0.006359	0.91	0.343
GENOTYPE	9	0.281225	0.031247	4.48	<.001
Water reg.GENOTYPE	9	0.249928	0.027770	3.98	<.001
Residual	76	0.530616	0.006982		

**Appendix Y. 1: The ANOVA of WUE for SCs of different wheat genotypes for across the field and glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_SC<sub>s</sub>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	5.66989	1.41747	44.60	
REP.*Units* stratum					
Water regime	1	1.34620	1.34620	42.35	<.001
GENOTYPE	9	1.65967	0.18441	5.80	<.001
Water reg.GENOTYPE	9	0.32767	0.03641	1.15	0.342
Residual	76	2.41562	0.03178		

**Appendix Z. 1: The ANOVA of WUE for PCs of different wheat genotypes across the field and glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: WUE\_PC<sub>s</sub>

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	4	19.99006	4.99751	69.53	
REP.*Units* stratum					
Water regime	1	0.85282	0.85282	11.87	<.001
GENOTYPE	9	2.75129	0.30570	4.25	<.001
Water reg.GENOTYPE	9	1.22401	0.13600	1.89	0.066
Residual	76	5.46257	0.07188		

**Appendix AA. 1: The ANOVA of PCs of different wheat genotypes for the selected 10 wheat genotypes grown in the glasshouse as influenced by water availability (25%FC) and (75%FC)**

Variate: PCs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	90040.	45020.	5.52	
REP.*Units* stratum					
TRT	1	16898533.	16898533.	2073.55	<.001
ENTRY	9	3741453.	415717.	51.01	<.001
TRT.ENTRY	9	3403216.	378135.	46.40	<.001
Residual	38	309683.	8150.		

**Appendix AB. 1: The ANOVA of PCs of different wheat genotypes for the selected 10 wheat genotypes in the field as influenced by water availability (25%FC) and (75%FC)**

Variate: PCs

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	135125.	135125.	0.75	
REP.*Units* stratum					
Water regime	1	8623124.	8623124.	47.60	<.001
Genotype	9	5790124.	643347.	3.55	0.010
Water regime. Genotype	9	3568861.	396540.	2.19	0.072
Residual	19	3441790.	181147.		

**Appendix AC. 1:** Ranking of genotypes using data normalized by the average between the field and glasshouse experiments.

Genotypes	GY	SB	RB	PB	R:S	SCC	RCC	GCS	SCS	RCS	PCS	WUE								mean
												GY	SB	RB	PB	GCS	SCS	RCS	PCS	
<b>BW152</b>	10	10	9	10	7	7	4	2	10	8	10	10	10	7	10	3	10	8	8	<b>8,1</b>
<b>LM71</b>	9	9	7	9	5	10	2	10	9	3	9	9	8	5	9	10	8	4	10	<b>7,6</b>
<b>LM48</b>	8	8	10	8	10	5	1	9	7	9	7	6	4	8	5	9	4	6	7	<b>6,9</b>
<b>BW140</b>	7	1	2	1	4	3	7	7	1	2	1	8	5	3	4	7	5	3	4	<b>3,9</b>
<b>LM70</b>	6	5	3	5	6	1	9	3	3	6	3	4	3	4	3	2	3	5	2	<b>4,0</b>
<b>BW162</b>	5	4	1	3	1	8	5	4	6	1	2	3	2	1	2	5	2	1	3	<b>3,1</b>
<b>BW141</b>	4	6	5	6	2	2	6	5	5	5	4	5	6	6	7	8	6	7	5	<b>5,3</b>
<b>LM26</b>	3	3	6	4	3	4	10	6	2	10	6	7	7	9	8	4	7	10	6	<b>6,1</b>
<b>LM47</b>	2	7	8	7	9	6	3	8	8	7	8	2	9	10	6	6	9	9	9	<b>7,0</b>
<b>LM75</b>	1	2	4	2	8	9	8	1	4	4	5	1	1	2	1	1	1	2	1	<b>3,1</b>

\*Ranked from overall best to least. Note: The ranking of genotypes was done after averaging the parameters under both stressed and non-stressed conditions.

### Appendix AD. 1: The ANOVA of CO<sub>2</sub>-C evolved by different wheat residues

Variate: CO<sub>2</sub>-C (mg kg<sup>-1</sup> soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.099E+01	3.049E+01	4.41	
Rep.*Units* stratum					
Treatment	1	1.142E+06	1.142E+06	1.651E+05	<.001
Variety	4	2.357E+05	5.892E+04	8516.89	<.001
Day	12	1.764E+05	1.470E+04	2124.49	<.001
Treatment.Variety	4	7.174E+04	1.793E+04	2592.30	<.001
Treatment.Day	12	6.598E+04	5.498E+03	794.76	<.001
Variety.Day	48	6.707E+04	1.397E+03	201.98	<.001
Treatment.Variety.Day	48	2.976E+04	6.200E+02	89.63	<.001
Residual	258	1.785E+03	6.918E+00		

### Appendix AE. 1: The ANOVA of cumulative CO<sub>2</sub>-C evolved by different wheat residues

Variate: Cumulative CO<sub>2</sub>-C (mg kg<sup>-1</sup>soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	6.607E+03	3.303E+03	81.57	
Rep.*Units* stratum					
Treatment	1	6.051E+07	6.051E+07	1.494E+06	<.001
Variety	4	1.234E+07	3.085E+06	76184.83	<.001
Day	12	9.180E+07	7.650E+06	1.889E+05	<.001
Treatment.Variety	4	4.708E+06	1.177E+06	29063.41	<.001
Treatment.Day	12	1.430E+07	1.192E+06	29422.14	<.001
Variety.Day	48	3.122E+06	6.504E+04	1606.06	<.001
Treatment.Variety.Day	48	8.403E+05	1.751E+04	432.31	<.001
Residual	258	1.045E+04	4.050E+01		

### Appendix AF. 1: The ANOVA of NH<sub>4</sub><sup>+</sup>-N released by different wheat residues

Variate: NH<sub>4</sub>-N (mg kg<sup>-1</sup> soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.2647	0.6323	3.23	
Rep.*Units* stratum					
Treatment	1	2134.6183	2134.6183	10914.86	<.001
Variety	4	4503.9584	1125.9896	5757.48	<.001
Day	12	8284.1883	690.3490	3529.93	<.001
Treatment.Variety	4	1978.8744	494.7186	2529.63	<.001
Treatment. Day	12	593.0853	49.4238	252.72	<.001
Variety. Day	48	928.4446	19.3426	98.90	<.001
Treatment.Variety. Day	48	977.8582	20.3720	104.17	<.001
Residual	258	50.4570	0.1956		



### Appendix AG. 1: The ANOVA of NO<sub>3</sub><sup>-</sup>-N released by different wheat residues

Variate: NO<sub>3</sub>-N (mg kg<sup>-1</sup> soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0549	0.0275	0.12	
Rep.*Units* stratum					
Treatment	1	2256.3999	2256.3999	10196.93	<.001
Variety	4	2894.1115	723.5279	3269.71	<.001
Day	12	23359.6881	1946.6407	8797.09	<.001
Treatment. Variety	4	1483.2402	370.8101	1675.73	<.001
Treatment. Day	12	1041.8733	86.8228	392.36	<.001
Variety. Day	48	2097.3294	43.6944	197.46	<.001
Treatment.Variety. Day	48	1503.9056	31.3314	141.59	<.001
Residual	258	57.0908	0.2213		

### Appendix AH. 1: The ANOVA of net N mineralized released by different wheat residues

Variate: N Mineralized (mg kg<sup>-1</sup> soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0137	0.5068	1.17	
Rep.*Units* stratum					
Treatment	1	8780.3473	8780.3473	20272.82	<.001
Variety	4	13923.7156	3480.9289	8037.07	<.001
Day	12	39490.6105	3290.8842	7598.28	<.001
Treatment. Variety	4	6771.7240	1692.9310	3908.79	<.001
Treatment. Day	12	2274.8528	189.5711	437.70	<.001
Variety. Day	48	3533.9083	73.6231	169.99	<.001
Treatment.Variety. Day	48	3319.2753	69.1516	159.66	<.001
Residual	258	111.7422	0.4331		

### Appendix AI. 1: The ANOVA of net P mineralized released by different wheat residues

Variate: P (mg kg<sup>-1</sup> soil)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.04875	0.02437	1.24	
Rep.*Units* stratum					
Treatment	1	185.54103	185.54103	9423.66	<.001
Variety	4	47.28843	11.82211	600.45	<.001
Day	12	385.22847	32.10237	1630.49	<.001
Treatment.Variety	4	25.64758	6.41190	325.66	<.001
Treatment. Day	12	78.98883	6.58240	334.32	<.001
Variety. Day	48	2.70387	0.05633	2.86	<.001
Treatment.Variety. Day	48	5.48326	0.11423	5.80	<.001
Residual	258	5.07972	0.01969		

